STRUCTURAL INVESTIGATIONS ON PLANT POLYSACCHARIDES, WITH PARTICULAR REFERENCE TO SOYBEANS

by

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ABSTRACT OF THESIS

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Title of Thesis Structural Investigations on Plant Polysaccharides, with particular reference to Soybeans.

Soybean hulls have furnished a series of acidic polysaccharides (I, II, IV and V), a xylan, a small amount of a "mannan" and cellulosic polysaccharides when extracted with various extractants.

The xylan contained xylose residues as the sole neutral sugar with a small amount (ca) 4% of glucuronic acid. The permethylated xylan gave, as the major cleavage product, 2,3 di-O-methyl-D-xylose together with small amounts of 2,3,4 tri-, 2- and 3-O-methyl-D-xylose and a partially methylated aldobiouronic acid, 2-O-(2,3,4 tri-O-methyl-D-glucuronic acid)-3-O-methyl-D-xylose. The structural significance of these results is discussed and the structural features compared with those of other xylans.

A preliminary examination has been carried out on the "mannan" and the cellulosic polysaccharides.

Acidic polysaccharides from soybean hulls (I, II, IV, V) gave the same major oligosaccharides on small-scale partial depolymerisation experiments, suggesting similarities between them.

Combined acidic polysaccharides I and II gave a number of acidic sugars on partial acid hydrolysis which were identified as 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose, O-(α-D-galactopyranosyluronic acid)-(1→2)-O-β-L-rhamnopyranosyl-(1→4)-O-(α-D-galactopyranosyluronic acid)-(1→2)-L-rhamnose, O-(D-galactopyranosyluronic acid)-(1→4)-O-(α-D-galactopyranosyluronic acid) (1→2)-L-rhamnose, 4-O-(α-D-galactopyranosyluronic acid)-D-galactopyranosyluronic acid, O-(α-D-galactopyranosyluronic acid)-(1→4)-O-(α-D-galactopyranosyluronic acid)(1→4)-D-galactopyranosyluronic acid, 6-O-(β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(β-D-glucopyranosyluronic acid)-D-galactose, 4-O-(β-D-glucopyranosyluronic acid)-L-fucose, 2-O-(β-D-glucopyranosyluronic acid)-D-mannose.

Partial acetolysis of acidic polysaccharides IV and V from soybean hulls and the acidic polysaccharide complex from soybean meal have yielded mixtures of neutral and acidic oligosaccharides amongst which the following have been identified, although not completely from each polysaccharide:- O-β-D-galactopyranosyl-[(1→4)-O-β-D-galactopyranosyl]$_n$-(1→4)-D-galactose, 2-O-β-D-galactopyranosyl-D-xylose, 2-O-α-L-fucopyranosyl-D-xylose, 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose, O-(α-D-galactopyranosyluronic acid)(1→2)-O-L-rhamnopyranosyl-(1→2)-L-rhamnose, O-(α-D-galactopyranosyluronic acid)(1→2)-O-β-L-rhamnopyranosyl (1→4)-O-(α-D-galactopyranosyluronic acid)(1→2)-L-rhamnose, and O-(D-galactopyranosyluronic acid)(1→4)-O-(α-D-galactopyranosyluronic acid)(1→2)-O-L-rhamnopyranosyl-(1→2)-L-rhamnose.

Gas/

Use other side if necessary.

Gas-liquid chromatographic evidence is given for the cleavage products from methylated acidic polysaccharides IV and V.

The structural features of these acidic polysaccharides have been discussed and compared with pectic acids, tragacanthic acid and other plant gums.

TO MY PARENTS

AND LOUISE.

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GENERAL INTRODUCTION.

INTRODUCTION

The soybean, or more correctly in botanical nomenclature Glycinemax, is classified as a member of the Leguminosae family whose members are more familiar to us in the form of the garden pea or haricot bean. There is a marked similarity between these species in size and shape although about 1,500 varieties of the soybean are known varying in their size and their colour from yellow to black.

The first mention of the soybean in recorded literature dates back to 3,000 B.C. in the old Chinese "Materia Medica" and it is in eastern countries that it is used still as a staple food. More recently, it has been introduced into North America where it has rapidly gained in popularity, making the United States of America the world's leading producer of the bean with a harvest of 752 million bushels in 1961-62 compared with only 5 million bushels in 1924-25.

Commercially, the crop is grown for the high percentage of oil present which, being easily extracted, makes it one of the leading produces of vegetable oil for the food, paint and printing ink industries. The residual meal can either be used as a cattle feed or a highly nutritious flour containing twice the protein found in other leguminous flours and four times that from cereal flours. Correspondingly the

carbohydrate content is relatively low. More recent uses of the meal are in the manufacture of adhesives, plastics and textile fibres.

A detailed analysis of the components present in soybeans has, so far, been limited to the items of commercial importance, namely the oil (1) and protein (2). The amino-acid composition of total protein has been analysed (3) but no protein structure has been derived. The carbohydrates have only been given a very limited analysis.

A characteristic feature of leguminous plants is their method of incorporating nitrogen, which is essential in the synthesis of protein and so, ultimately in the growth and reproduction of the species. The majority of plants abstract nitrogenous materials from the surrounding soil but these plants use the nitrogen present in the atmosphere. Phosphorus is also an important constituent for plant life as phosphorylated sugars are major units in nucleotide pools. Some measurements have been carried out by Togari (4) on the content of total sugar, starch, phosphorus and nitrogen at various stages of the soybean's life cycle. All four components show a slow increase during the preflowering stage, but the total sugar and starch present shows a marked increase during flowering and reaches a maximum

shortly after flowering. Other workers (5) have noted that the reducing sugar and total sugar content shows a decrease with age, but to counteract this, there is an increase in the pentosan and galactan present. This evidence suggests that these are reserve poly-saccharides and are synthesised from smaller units.

The reducing disaccharide, maltose, has been found (6, 7) as a component in the aqueous ethanol extract from germinated seeds, being present in amounts ranging from 14% in the roots to 45% in the petioles. During storage of the germinated seeds, maltose vanishes completely.

Other oligosaccharides have been isolated and studied in some detail. Kawamura (8, 9) has calculated that dehulled and defatted soybean meal, of which about 30% is non-protein material, contains 7.46% oligosaccharides consisting of the non-reducing sugar, sucrose, raffinose and the tetrasaccharide, stachyose. Later studies have also shown the presence of the related pentasaccharide, verbascose (7, 10). These oligosaccharides have the general structure of:-

$$[\alpha\text{-}\underline{\underline{D}}\text{-Gal p } 1\rightarrow 6]_n\text{-}\alpha\text{-}\underline{\underline{D}}\text{-G p}1\rightarrow 2\text{-}\beta\text{-}\underline{\underline{D}}\text{-Fru f}$$

where $\underline{\underline{D}}$ - Gal p is $\underline{\underline{D}}$ - galactopyranosyl

D - G p is D - glucopyranosyl

D - Fruf is D - fructofuranosyl

and n = O for sucrose, n = 1 for raffinose, n = 2 for stachyose and n = 3 for verbascose.

This family of oligosaccharides is found extensively in nature and particularly so in the Leguminosae (11). Many views have been put forward to explain their biosynthesis. It is known that the pods are entirely free from these sugars and that they are also the last carbohydrate formed in the seed. When germination occurs, they are the first carbohydrate used up and so would appear to act as a nutrient to the young seedlings. There, no stachyose or raffinose is found (12) and fructose and glucose take their place. Healthy raw seeds show virtually no change in these sugars. The absence of free galactose in the seedlings points to the rapid use of this sugar in the breakdown of the raffinose family. Artificial increase in the temperature or humidity of the plant's environment also causes breakdown of the raffinose series but here produces free galactose.

Soybeans exhibit enzyme activity of the following types (13):- α- galactosidase, galactokinase, U.D.P. -

glucosepyrophosphorylase, U.D.P. - galactopyrophosphorylase, U.D.P.-gluco-galactophosphate transferase, and U.D.P. - glucose - 4 - epimerase. In all cases, U.D.P. stands for the uridine diphospho - group. It is quite reasonable to suggest that a combination of some or all of these enzymes causes the raffinose family or galactose - 1-phosphate, acting as potential α-D-galactopyranosyl donors, to form either galactan chains or the side chains of galactomannans.

The longer chain carbohydrates vary with the state of the growth of the plant. A starch, which is produced during the germination of the bean, has been isolated by Sasaki (14). There is no indication whether maltose (4-o α-D- glucopyranosyl - D-glucose), which is, as earlier stated, also produced during germination is a precurser for use in starch synthesis or is a degradation product. This starch, which is present in amounts up to 7.7% has a saccharification value midway between those of potato and wheat starches. More recent studies on the crystallinity of starch from the seedlings has shown a great variation with the prevailing temperatures of germination. There was, however, no difference in the starches isolated from the cotyledons and hypocotyls from the same seedlings (15). It is significant to note that starch (16), as well as maltose, is completely absent from the mature bean.

The subsequent extraction of other polysaccharides from dehulled and defatted soybean meal is hindered by the abnormally high percentage of protein present. Normal polysaccharide extraction schemes cannot be directly applied as large amounts of protein are extracted simultaneously and cannot easily and conveniently be separated from the polysaccharide components. Kawamura (17) has used a procedure where themeal is pretreated with a 0.2% solution of sodium hydroxide. This treatment effectively solubilised most of the protein and did not appear to remove any carbohydrate or alter the composition of the residual material in any way. The same school (18, 19) have then used conventional extraction procedures on the pretreated meal, isolating several polysaccharide fractions. An extraction with hot water has yielded a fraction which gives arabinose, galactose, xylose and galacturonic acid on hydrolysis. Subsequent extractions of hot water extracted meal with 0.5% ammonium oxalate solution and 0.2 to 15% sodium hydroxide have given further fractions. After purification with Fehling's solution these polysaccharides were found to give fucose, rhammose, xylose, arabinose, galactose and galacturonic acid on hydrolysis. These investigations have not been elaborated on, and so no criterion of homogeneity is available.

There is also no evidence about the structural features of these fractions.

The problem of the high protein concentration is not found in the hulls of the bean, allowing isolation and fairly extensive structural evaluation of the polysaccharides to be readily carried out. Whistler and Saarnio (20) have isolated a galactomannan from defatted soybean hulls in a 2% yield by extraction with water at 40°. After precipitation *via* its copper complex, the galactose: mannose ratio on hydrolysis was 2.1 : 3, while the methylated galactomannan gave on hydrolysis, 2, 3, 4, 6 tetra -O- methyl - D-galactose (2 moles), 2, 3, 6 tri -O- methyl -D-mannose (1 mole) and 2, 3 di -O- methyl -D- mannose (2 moles). These results suggest that the polysaccharide has a structure of a backbone of 1, 4 linked -D-manno-pyranose residues to which are attached single unit side chains of 1, 6 linked ∝ -D-galactopyranose units.

This proposed structure for soybean hull galacto-mannan is similar to those of galactomannans found in a wide variety of plants and especially those of the *Leguminosae*. All these galactomannans are further characterised by small differences in their fine structure. The differences have been classified and compared in a review by Smith and Montgomery (21).

Two papers have been published which give information about xylans from soybean sources. Whistler and Sanella (22) have extracted water-extracted hulls with alkali. A polysaccharide fraction, in 11% yield, was isolated on acidification of the extract and gave xylose as the sole neutral sugar on hydrolysis but the polysaccharide was not examined further. Addition of ethanol to the supernatant liquid gave a further fraction in 6% yield. After repeated precipitations with ethanol, the polysaccharide was electrophoretically homogeneous. The presence of 4 - O - methyl -D-glucuronic acid residues on the polysaccharide was shown by the isolation of an aldobiouronic acid, which was characterised by derivative formation.

The polysaccharide obtained above was methylated and gave the follow sugars on hydrolysis:- 2, 3, 4 tri -O- methyl- D-xylose, 2, 3, 5 tri -O- methyl -L-arabinose, 2, 3, 4, 6 tetra -O- methyl -D- galactose, 2, 3 di-O- methyl -D-xylose, 2, 3 di -O- methyl - D-glucose and 2-O - methyl -D- xylose.

The structure of this polysaccharide is evidently very complex. The presence of 2, 3 di-O-methyl- xylose residues from the methylated polysaccharide indicate that

it belongs to the xylan group as the basal chain must consist of 1, 4 linked xylose units. Also, D-xylopyranose, D-galactopyranose and L-arabinofuranose units are present as non-reducing end groups. 4-O-Methyl- D-glucuronic acid is linked to C-2 of some xylose units and further branch points also occur at C-3 of other xylose residues. The role of D-glucose must remain obscure. The polysaccharide needs further investigation, with, for example, the formation of netural oligosaccharides from fragmentation techniques, before a firm conclusion can be drawn about its fine structure.

Another xylan has been isolated from soybean stems (23) by alkaline extraction and subsequent acidification of the extract. This xylan is devoid of arabinose residues and has a uronic anhydride content of 9%. Paper chromatographic evidence from the hydrolysate of the polysaccharide suggests that the acidic sugar present is 4-O-methyl -D-glucuronic acid.

The present investigation forms part of a project sponsored by the United States Department of Agriculture under the title:- "A quantitative study of the polysaccharides in fat-free soybean meal to provide information needed to improve the processing of meal for food and feeds, thereby contributing to its expanded utilisation".

The first part of the project has been concerned with

further investigation of hull components. Defatted hulls were extracted with water at room temperature and 60° (24). The two extracts were each fractionated by the use of copper acetate into neutral components, Galactomannans I and II, and two acidic components. The galactomannans differed in the ratio of galactose: mannose formed on hydrolysis, galactomannan I having a ratio of 1:1·4 and galactomannan II one of 1:2.35. Methylation analysis of each polysaccharide confirmed the earlier basal structure put forward by Whistler and Saarnio (20). Fragmentation of the polysaccharides by partial acetolysis allowed characterisation of polymer homologous oligosacoharides of the general structure (I)

$$\beta\text{-}\underline{\underline{D}}\text{-Man}\,p\,[\,1\rightarrow 4\,\beta\text{-}\underline{\underline{D}}\text{-Man}\,p\,]_n\,1\rightarrow 4\,\underline{\underline{D}}\text{-Man}$$

where n = 0, 1, 2 and 3.

I.

as well as the two oligosaccharides (II) and (III)

$$\alpha\text{-}\underline{\underline{D}}\text{-Gal}\,p\,1\rightarrow 6\,\underline{\underline{D}}\,\text{Man} \qquad \text{(II)}$$

$$\alpha\text{-}\underline{\underline{D}}\text{-Gal}\,p\,1\rightarrow 6\,\beta\text{-}\underline{\underline{D}}\text{-Man}\,p\,1\rightarrow 4\,\underline{\underline{D}}\,\text{Man} \qquad \text{(III)}.$$

The isolation of these oligosaccharides gives definite evidence for the structures of the galactomannans consisting of α-**D**-galactopyranosyl residues linked to a chain of 1, 4 linked β-**D**-mannopyranosyl residues through C-6 of the mannose units. This structure is very similar to those of

other galactomannans isolated from leguminous sources which are exemplified by guaran (25) and locust bean gum (26).

Water-extracted hulls have been extracted with 0.5% ammonium oxalate solution (27). After precipitation as its calcium salt, an Acidic Polysaccharide III was obtained (Acidic polysaccharides I and II were extracted in conjunction with Galactomannans I and II respectively). This polysaccharide, which was electrophoretically homogeneous and was eluted as a single band on diethylaminoethylcellulose chromatography, belonged to the pectic group. The high uronic acid anhydride content (76%) allowed high yields of acidic oligosaccharides to be obtained *via* partial acid hydrolysis. Among the oligosaccharides obtained, 2-O- (α - D-galactopyranosyl uronic acid) -L-rhamose, 4 - O - (α-D- galactopyranosyl uronic acid) - D- galactopyranosyl-uronic acid and the polymer homologous trisaccharide have been characterised.

Preliminary examination of the cleavage products from the methylated acidic polysaccharide by gas-liquid chromatography has indicated the presence of large amounts of 2, 3, 6 tri -O- methyl-galactose, suggesting that chains of 1, 4 linked galactose residues are integral parts of the

structure.

The present investigation concern the structure of further polysaccharide fractions from the hull cell-wall, together with the characterisation of oligosaccharides formed by fragmentation of the acidic polysaccharide complex obtained from soybean meal.

GENERAL METHODS OF INVESTIGATION.

GENERAL PROCEDURES.

Paper Chromatography. Qualitative and quantitative chromatograms were run on Whatman No. 1 paper. Large scale separations were carried out on Whatman No. 3MM filter sheets which had previously been washed with water.

Chromatography Solvent Systems (v/v)

(A) Ethyl Acetate : Pyridine : Water (10 : 4 : 3)

(B) Ethyl Acetate : Acetic Acid : Formic Acid : Water (18 : 3 : 1 : 4)

(C) Ethyl Acetate : Acetic Acid : Formic Acid : Water (18 : 8 : 3 : 9)

(D) Benzene : Butan -1-ol : Pyridine : Water (1 : 5 : 3 : 3, upper layer).

(E) Butan -1-ol : Ethanol : Water (4 : 1 : 5, upper layer)

(F) Butan -2-one. 50% sat. Water : 1% ammonia

(G) Butan -1-ol : Ethanol : Water (1 : 1 : 1)

(H) Butan -2-one : Acetic Acid : Water, saturated Boric Acid (9 : 1 : 1)

(I) Ethyl Acetate : Pyridine : Acetic Acid : Water (5 : 5 : 1 : 3)

(J) Butan -1-ol : Acetic Acid : Water (4 : 1 : 5, upper layer)

(K) Butan -1-ol : Ethanol : Water (4 : 1 : 1, upper layer)

(L) Butan -1-ol : Ethanol : Water : Ammonia (40 : 10 : 49 : 1, upper layer)

(M) Butan -2-one : Water : Ammonia (200 : 17 : 1)

The R_X value of a sugar refers to its rate of mobility relative to sugar X.

The R_G value of a methylated sugars refers to its rate of mobility relative to 2, 3, 4, 6 tetra -O- methyl - D- glucose.

Paper Ionophoresis. Paper electropheretograms were run in borate buffer at pH 10 (28) for four hours at a potential voltage of 350 v. The M_G value of a sugar refers to its rate of mobility relative to D- glucose, a correction beingmmade for electro-endosmotic flow by incorporating a standard of 2, 3, 4, 6 tetra -O- methyl -D- glucose.

Chromatographic Spray Reagents.

(A) p-Anisidine hydrochloride (29). This spray was used for reducing sugars.

(B) Aniline oxalate. Certain methylated sugars were detected by this spray.

(C) Silver nitrate (30). Non-reducing sugars, sugar glycosides and sugar alcohols were identified with this spray.

(D) Hydroxylamine - Ferric Chloride (31). Lactones and esters gave mauve or violet spots.

(E) Triphenyltetrazolium salt (30). Reducing sugars only gave bright red spots if unsubstituted on position C-2 .

(F) Periodate-permanganate reagent (32). Sugar alcohols gave yellow spots on pink background.

(G) Periodate - Schiff's reagent (33). Sugars releasing formaldehyde gave purple spots, those releasing malonaldehyde derivatives gave yellow spots, while other aldehydes produced gave blue spots.

Column Chromatography

Charcoal - Celite Column (34).

Charcoal (B.D.H. or M. and B. brands) was washed free of acid by repeated washing with boiling water. Celite was washed first with 1 : 1 hydrochloric acid and then washed free of acid with distilled water. A slurry composed of an equal weight of charcoal and Celite in water was poured into a column plugged at one end with paper pulp and allowed

to settle under gravity. The packing surface was protected with a filter paper. Before use, the column was prewashed with the greatest concentration of eluant to be used and finally washed well with a large volume of distilled water (about 50 bed volumes).

DEAE Cellulose Columns. (35)

Diethylaminoethyl cellulose was washed three times alternatively with 0.5M Hydrochloric acid and 0.5M sodium hydroxide. After washing with water, the slurry was applied to a column plugged with glass wool. The column was generated in the desired phosphate form by elution with a 0.5M buffer of sodium dihydrogen phosphate at pH 6·1. Further equilibration was achieved by elution with a large volume of 0.005M phosphate buffer. The polysaccharide was applied in a minimum volume and eluted with the desired buffers. A flow rate of 60 ml. per hour was maintained for most purposes.

DEAE Sephadex Columns.

Diethylaminoethyl - Sephadex - A25 was initially swollen in water and fines removed by decantation. The resin was washed alternatively with 0.5M hydrochloric acid and 0.5M sodium hydroxide before generating in the formate

form by washing with 0.5M formic acid. The resin was packed in a column plugged with glass wool and equilibrated with 0.005M formic acid. During elution of the column, a flow rate of 60 ml. per hour was maintained.

Gas-Liquid Partition Chromatography.

Qualitative gas-liquid chromatography was carried out in a "Pye Argon" instrument using the following columns:-

(a) 15% by weight of butan -1, 4- diol succinate polyester on dichloro-dimethylsilane treated Celite (80-100 mesh) at 175°.

(b) 10% by weight of polyphenyl ether [m-bis-(m-phenoxy-phenoxy) benzene] on dichloro-dimethylsilane treated Celite at 200°.

(c) 3% by weight of neopentylglycol adipate polyester on dichloro-dimethylsilane treated Celite at 150° or 175°.

Retention times (T) of methyl glycosides are given relative to the mobility of methyl 2, 3, 4, 6 tetra -O- methyl-β- D - glucopyranoside. Incompletely resolved values are given in parenthesis.

HYDROLYSIS

(A) Hydrolysis with Sulphuric Acid. Samples of polysaccharides

(5 - 10 mg.) or oligosaccharides (1 - 2 mg.) were hydrolysed by heating at 100° for varying times with sulphuric acid of given normality (1 - 2 ml.). The cooled solutions were neutralised with barium carbonate, filtered and deionised with Amberlite resin IR 120 (H) before concentrating.

(B) Hydrolysis with Hydrochloric Acid. Samples of methylated polysaccharides (1 - 5 mg.) and oligosaccharides (1 - 2 mg.) were hydrolysed with 3% hydrochloric acid (1 - 2 ml.) for varying times. The cooled solutions were neutralised with silver carbonate, filtered through glass fibre paper and concentrated.

(C) Methanolysis. Samples of methylated oligasaccharides (0.2 - 2 mg.) were heated at 100° for varying times with 4% methanolic hydrogen chloride. The methyl glycosides solution was neutralised with silver carbonate, filtered and concentrated to a small volume in the presence of chloroform.

Small-scale Methylations (36) were carried out by dissolving the oligosaccharide (0.5 - 2 mg.) in N,N dimethylformamide (0.2 ml.) and adding methyliodide (0.2 ml) and silver oxide (0.2 g.) The mixture was vigorously shaken for 18 hr. in the dark at room temperature. After

filtering, the solution was evaporated to dryness by repeated distillation under reduced pressure in the presence of toluene.

Demethylations (37). The sugar (0.2 - 5 mg.) was dissolved in dry dichloromethane (1 - 2 ml.) and cooled to -80° in liquid nitrogen. Similarly cooled boron trichloride (1 - 2 g.) was added and the solution left at -80° for 30 min. before allowing to reach room temperature. The solution was kept at 18° for 16 hr. under anhydrous conditions and some remaining solvent was evaporated off. Boric acid remaining was removed as the volatile methyl borate by repeated distillation with methanol.

Borohydride Reduction. Oligosaccharides (2 - 3 mg.) were dissolved in water and left overnight and at room temperature with potassium borohydride. Excess borohydride was destroyed with Amberlite IR 120 (H) resin, filtered and solution evaporated to dryness. Boric acid was removed as methyl borate by repeated distillation with methanol.

Aniline Derivatives were prepared by refluxing equimolar portions of the sugar and freshly distilled aniline in ethanol for 2 hr. The reaction was carried out in the

dark in a stream of nitrogen. Excess solvent was evaporated off and the derivative recrystallised from an appropriate solvent.

Aldonolactones were prepared by dissolving the sugar in water and adding 0.5 ml. bromine. The mixture was set aside in the dark for 3 days with occasional shaking and then aereated to remove excess bromine. The solution was neutralised with silver carbonate, filtered, silver salts precipitated with hydrogen sulphide, filtered and concentrated. The syrup was extracted with acetone and crystallised from an appropriate solvent.

Aldonamides were prepared from the aldonoloctone by allowing the dry syrup to stand for 24 hr. at 4° with 2% methanolic ammonia. The solvent was evaporated off and the derivative crystallised out.

Phenylosazones were prepared by heating an aqueous solution of the sugar for 1 hr. at 100° in the presence of acetic acid (0.2 ml.), freshly distilled phenyl hydrazine (0.3 ml.) and 2 drops of saturated sodium bisulphite solution. The crystalline osazone was filtered off, dissolved through the filterpaper with ether to leave behind impurities and recrystallised.

Acetates were prepared by dissolving the sugar in pyridine and adding acetic anhydride. The solution was vigorously shaken in the dark for 18 hr. and poured into ice-water. The acetate precipitated out, was collected and recrystallised from an appropriate solvent.

Nitrobenzoyl derivatives were prepared by dissolving the sugar in pyridine to which was added p-nitrobenzoyl chloride. The mixture was kept at 60-70° for 30 min. before leaving overnight at room temperature. Saturated sodium hydrogen carbonate solution was added until no further effervescence was noted, diluted with water and extracted with chloroform. Crystals appeared on concentration which were recrystallised from a suitable solvent.

Methoxyl Contents were measured by the semi-micro Zeisel method (38)

Nitrogen Estimations were determined by the semi-micro Kjeldahl method

Optical Rotations were observed in aqueous solutions, at 20 ± 2°, unless otherwise stated.

Evaporations were carried out under reduced pressure at 40° or below.

Melting points were taken either in capillary tubes or on a Kofler hot-stage microscope and are uncorrected.

Estimation of Sugars by Phenol - Sulphuric Acid Method (39)

To the sample (10-70 g.) in water (1 ml), was added 5% aqueous phenol solution (1 ml.) and concentrated sulphuric acid (5 ml) was pipetted on to the surface. The solution was mixed, set aside for 10 mins and kept at 25-30° for 10 min. The intensity of colour developed was measured spectrophotometrically and compared with a standard graph, made using galactose as standard. The wavelength used was 490 mμ.

Estimation of Uronic Acid Anhydride by Carbazole Method (40)

The sample (5-100 g.) in water (1 ml.) was added to concentrated sulphuric acid (6 ml.) and heated at 100° for 20 minutes. After cooling, carbazole reagent (0.2 ml.) was added and the tubes were left for 2 hr. The colour developed was measured spectrophotometrically at 530 mμ and was compared with a standard graph using galacturonic acid.

Estimation of Uronic Acid Anhydride by Decarboxylation (41)

The uronic anhydride content of polysaccharides was measured from the amounts of carbon dioxide produced on reaction with 19% hydrochloric acid.

Estimation of Rhamnose Content of Oligosaccharides (42).

The Rhamnose content was measured by the L-Cysteine Hydrochloride method which gives a characteristic colour

at 400 mμ.

Estimation of Formaldehyde (43)

The formaldehyde released on periodate oxidation of oligosaccharides was measured with chromotropic acid reagent. A characteristic colour is obtained at 570 mμ and measurements are taken from a standard graph obtained by estimation of formaldehyde released by periodate oxidation of glucose.

Organic Solvents were purified and dried by methods quoted by Vogel (44);

Acetylation of Polysaccharides. (45) The polysaccharide (1 g.) was dispersed thoroughly in formamide (25 ml.) by vigorous agitation for 3 days at room temperature. Anhydrous pyridine (27 ml.) was added over 2 hours and then acetic anhydride (18 ml.) was added dropwise with stirring to the cold mixture over 4 hours. The acetylation was completed by vigorous agitation for a further 3 days.

The solution was poured into ice cold water (100 ml.) for neutral polysaccharides and ice-cold hydrochloric acid (2N, 200 ml) for acidic polysaccharides. Acetylated polysaccharide was removed at the centrifuge, washed with cold water and dried over phosphorus pentoxide. The solid was dissolved in chloroform, the solution dried over

anhydrous sodium sulphate, concentrated and precipitated by poring into light petroleum (B.P. 60-80°). After washing with light petroleum, the polysaccharide acetate was dried over phosphorus pentoxide and paraffin wax.

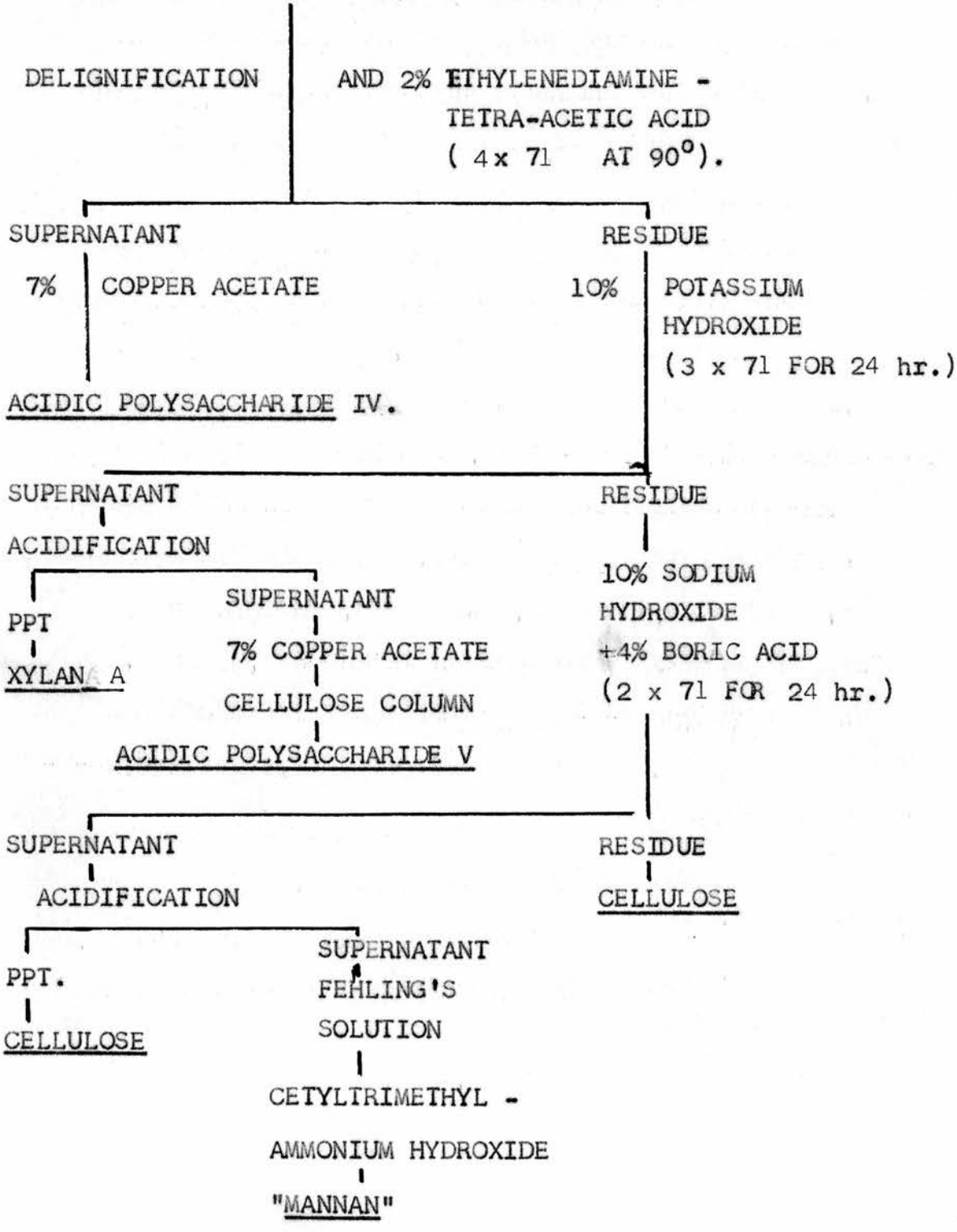
Quantitative Estimations of Sugars. These estimations were carried out by the procedure of Wilson (46) using aniline hydrogen phthallate.

DISCUSSION

EXTRACTION AND FRACTIONATION OF SOYBEAN HULL POLYSACCHARIDES.

The extraction scheme carried out on soybean hulls is summarised in the flow sheet on the following page. The acidic polysaccharide fractions which had been extracted from soybean hulls by water along with galactomannans I and II (24) were each fractionated further *via* their insoluble copper salts (47) into Acidic Polysaccharide I, $[\alpha]_D$ + 140° with uronic acid anhydride content of 39% from the cold water extract, and Acidic Polysaccharide II, $[\alpha]_D$ + 140° with uronic acid anhydride content of 36% from the hot water extract. Both polysaccharides gave an hydrolysis similar mixtures of galacturonic acid, galactose, mannose, arabinose, xylose, fucose, rhamnose and two methylated sugars with the chromatographic mobilities of 2-O-methylxylose and 2-O-methylfucose.

EXTRACTION OF SOYBEAN HULLS. - FLOW SHEET.



Ion-exchange chromatography of each polysaccharide on diethylaminoethylcellulose showed that although about 70% of each polysaccharide was eluted with phosphate buffers of the same concentration, four smaller fractions were also obtained.

Hulls which had been extracted with 0.5% ammonium oxalate solution (27) were delignified by the chlorine-ethanolamine procedure (48). Timell and John (49) have calculated that this procedure is less degradative to residual polysaccharides than the sodium chlorite process (50). Subsequent extraction with aqueous 2% unbuffered ethylenediaminetetra-acetic acid disodium salt gave Acidic Polysaccharide IV after purification *via* its copper salt. This polysaccharide, $[\alpha]_D$ + 65°, had a uronic acid content of 44% and gave on hydrolysis a similar mixture of sugars to these from Acidic Polysaccharide III, obtained from the ammonium oxalate extraction, namely galacturonic acid, galactose, arabinose, xylose, rhamnose, fucose and traces of 2-*O*-methylxylose and 2-*O*-methylfucose.

Ion-exchange chromatography of acidic polysaccharide IV on diethylaminoethylcellulose gave two discrete bands, eluted with 0.2- and 0.5*M*- phosphate buffers respectively. These bands both had similar optical rotations, uronic

acid anhydride contents and sugar compositions on hydrolysis. When the polysaccharide was saponified, however, it was eluted from the ion-exchange medium as a single band but only on elution with sodium hydroxide solution. It is likely that elution of the polysaccharide as two bands is caused by variations in the degree of esterification of the original polysaccharide.

Extraction of the hulls with 10% potassium hydroxide solution gave Hemicellulose 1 on acidification of the extract and Hemicellulose 2 on addition of ethanol to the residual solution. Subsequent extraction with a solution of 10% sodium hydroxide containing 4% boric acid (51) gave Hemicellulose 3 on acidification of the extract while addition of ethanol to the supernatant liquid gave Hemicellulose 4.

The main neutral sugar constituent of hemicellulose 1 was xylose. Fractionation of the polysaccharide *via* its insoluble copper complex (52) afforded a Xylan which had a low uronic acid anhydride content (*ca* 4%). Hydrolysis showed that xylose was the sole netural sugar formed and that glucuronic acid was the uronic acid.

Hemicellulose 2 proved to be heterogeneous when treated with copper acetate solution, two discrete

fractions, 2A and 2B, being obtained. The former fraction, 2A, appeared to be almost identical to the xylan obtained from hemicellulose 1 while the latter fraction, 2B, gave a polysaccharide which had a uronic acid anhydride content of 25%. Hydrolysis showed that the uronic acid present was galacturonic acid. Diethylaminoethyl-cellulose chromatography of the second fraction gave only one band but only about half the adsorbed polysaccharide was recovered from the column. A quantitative analysis of the major neutral sugars in the hydrolysates of the applied and the eluted polysaccharides showed that galactose, glucose, arabinose, fucose and rhamnose residues were present in the same ratio while xylose residues were present in substantially reduced proportions in the eluted polysaccharide. A xylan fraction was evidently being adsorbed irreversibly on the ion-exchange medium. This xylan could similarly be adsorbed on unmodified cellulose, Acidic Polysaccharide V, $[\alpha]_D$ + 140° (Found; uronic acid anhydride, 45%) being eluted with water. This polysaccharide was identical to that eluted from the ion-exchange medium; The xylan could not be recovered pure, even on extraction of the cellulose with alkali.

Hemicellulose 3 obtained by alkaline borate extraction

of the hulls was freed from other contaminating polysaccharides by solubilising these in alkali. The residual polysaccharide contained only glucose residues and was probably degraded cellulose which, although extracted under alkaline conditions, later became insoluble in alkali.

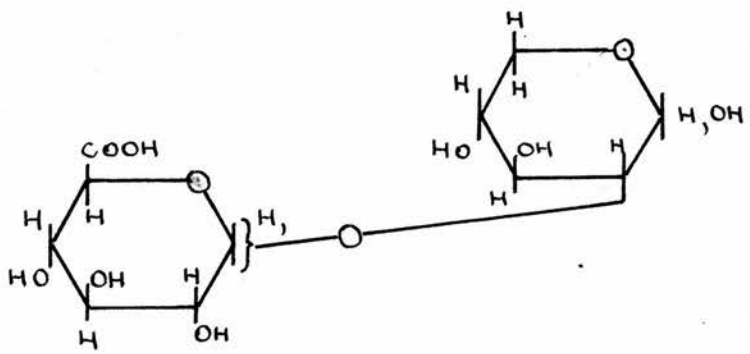
Hemicellulose 4 was separated into two fractions, *via* their insoluble copper complexes, which were found on hydrolysis to be mannose-rich and xylose-rich respectively. Further fractionation of the mannose-rich component with cetyltrimethylammonium hydroxide gave a "mannan", $[\alpha]_D$ - 31° which contained mannose, galactose and glucose residues in the ratio of 23 : 2 : 1.

The residue which remained insoluble throughout the extraction sequence gave mainly glucose on hydrolysis but also showed traces of mannose and xylose residues.

XYLAN A

Partial hydrolysis of the xylan with N-sulphuric acid gave a mixture of xylose and acidic oligosaccharides. The syrup was separated into neutral and acidic components by chromatography on diethylaminoethyl-Sephadex followed by separation of the acidic sugars by thick paper chromatography.

The major component of the acidic sugars was chromatographically identical to 2 - O - (α - D-glucopyranosyluronic acid) -D-xylose. Hydrolysis of the aldobiouronic acid gave glucuronic acid while the derived methyl ester methyl glycosides on reduction with borohydride and hydrolysis gave glucose and xylose in equimolar amounts. Gas-liquid chromatography of the methanolysis products from the methylated aldobiouronic acid gave peaks with the retention times of the methyl glycosides of 2, 3, 4 tri-O-methyl - glucuronic acid and 3, 4 di -O- methylxylose. These results are in agreement only with a structure of 2- O- (D-glucopyranosyluronic acid) -D-xylose (IV).



IV

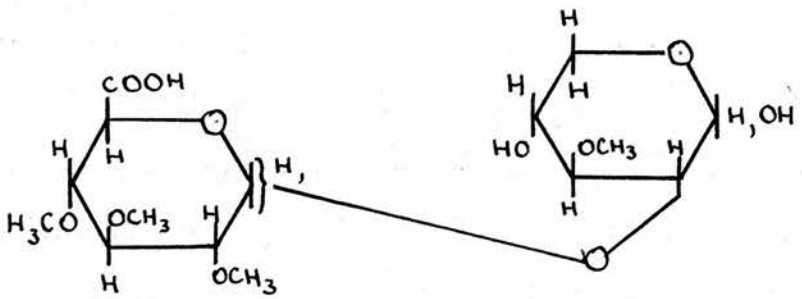
The other acidic sugars included glucuronic acid, two slow moving sugars which both gave glucuronic acid and xylose on hydrolysis, and a trace of a disaccharide which had the chromotographic mobility of 2 - O - (∝ - D - galacto-pyranosyluronic acid) -L- rhamnose.

The xylan was converted into its fully methylated derivative which gave a mixture of neutral and acidic sugars on hydrolysis. These sugars were separated by ion-exchange chromatography on diethylaminoethyl-Sephadex. The resultant mixture of neutral sugars was itself separated on a charcoal - Celite column, followed, where necessary, by separation on filter sheets. The methylated sugars present, all being characterised by crystalline derivative formation, were 2, 3, 4 tri-, 2, 3 di-,2- and 3-O- methyl -D-xylose in the molar ratio of 1 : 118 : 1.2 : 0.7.

The acidic sugar from the methylated polysaccharide was shown to be a partially methylated aldobiouronic acid (figure V), 2 - O - (2, 3, 4 tri -O-methyl -D-glucopyranosyl-uronic acid) -3-O-methyl -D-xylose on the basis of the following experiments. The derived methyl ester methyl glycosides were reduced

with borohydride and hydrolysed. The cleavage products were identified as 3 - O - methyl-xylose and 2, 3, 4 tri -O- methylglucose by paper chromatography, ionophoresis and gas-liquid chromatography of the derived methyl glycosides.

The major portion of the aldobiouronic acid was fully methylated, the methylester methyl glycosides were reduced with lithium aluminium hydride and the resulting neutral disaccharide was hydrolysed. The cleavage products were separated on filter sheets and characterised as 3, 4 di -O- methyl -D-xylose and 2, 3, 4 tri -O- methyl -D- glucose by the formation of crystalline derivatives.



V

The methylation data indicated that the xylan structure was based upon chains of 1, 4 linked D-xylopyranose units as shown in figure VI

$$\cdots\cdots 4\text{-}\underline{D}\text{-}\mathrm{Xyl}\ p\ 1\rightarrow 4\text{-}\underline{D}\text{-}\mathrm{Xyl}\ p\ 1\rightarrow 4\text{-}\underline{D}\text{-}\mathrm{Xyl}\ p\ 1\cdots\cdots$$

VI

The isolation of tri -O- methyl -D-xylose is evidence that xylose residues also terminate the main chain at the non-reducing end. Several explanations are possible for the appearance amongst the cleavage products of small amounts of the two mono-methylxyloses. They may have arisen either from under-methylation of the polysaccharide or from demethylation during hydrolysis. These sugars would also arise if the main chain was branched at C - 2 or C - 3 of certain xylose residues while 3 - O - methylxylose could also arise from hydrolysis of the aldobio-uronic acid formed.

The isolation of the partially methylated aldobiouronic acid (figure V) on hydrolysis of the methylated xylan shows certain other features of this xylan. The position of linkage of the uronic acid occurs at C - 2 of certain xylose residues and the uronic acid

is also linked directly to the xylan backbone, otherwise a fully methylated aldobio-uronic acid would have been isolated.

The occurrence of the aldobiouronic acid IV on the hydrolysate of the xylan is further evidence of the position of linkage being to C-2 of the xylose residues and also confirms that the acidic sugar is glucuronic acid and not its 4-methyl ether.

The polysaccharide has a high negative optical rotation which suggests that the glycosidic linkage between two adjacent xylose residues has a β configuration. The combination of all these observations leads to the structure of the xylan from soybean hulls being that given in figure VII

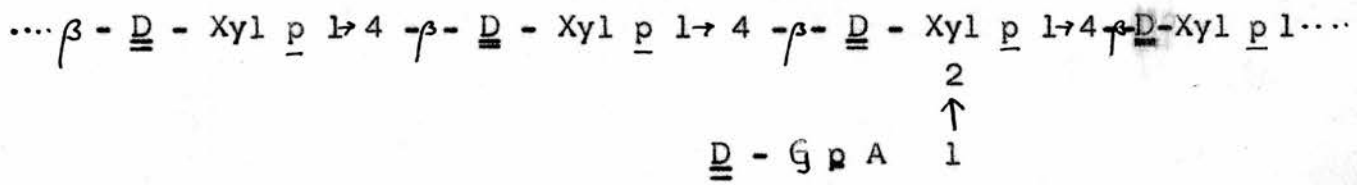


Figure VII

The observed molecular weight of the xylan corresponds to a degree of polymerisation of 72± 4. Although the average chain length based on methylation end-group assay is about 120, this value cannot be

regarded as correct as 2, 3, 4 tri -O-methylxylose and its methyl glycosides are highly volatile (53) and would be lost, in part at least, during the working up of the hydrolysate from the methylation. Until some more exact method is possible for end-group assay, it is not possible to say whether this xylan is linear or has a small degree of branching.

The structures of different xylans isolated from land plants have been reviewed by Aspinall (54) and it is possible to give a comprehensive pattern which incorporates the more common features which have been found. The structure in figure VIII gives these features.

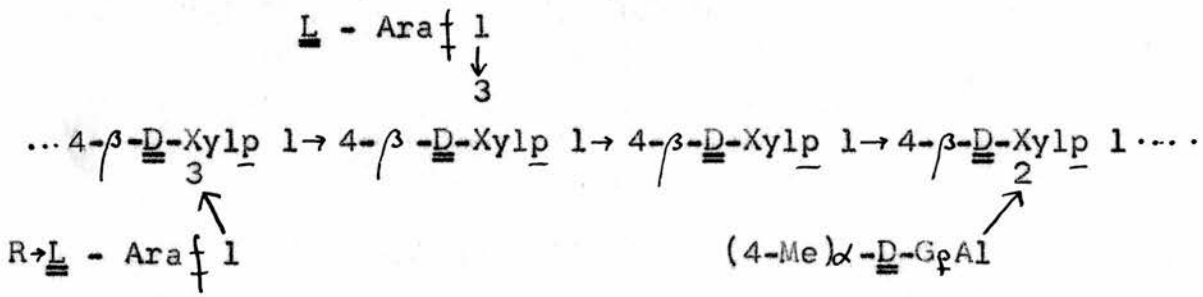


Figure VIII

The basic feature of these polysaccharides has been known for many years to be that of a 1, 4 linked polymer

of D-xylopyranose residues from the pioneer work of Haworth, Hirst and Percival (55, 56) on esparto grass xylan. Further proof of the position of linkage and that the glycosidic linkage between two adjacent xylose residues had a β configuration came from partial acid hydrolysis studies on corn cob xylan when the series of oligosaccharides (Figure IX) was isolated (57).

$$\beta\text{-D-Xyl } \underline{p}\ 1\rightarrow[\ 4\text{-}\beta\text{-D-Xyl } \underline{p}\ 1]_n \rightarrow 4\text{-D-Xyl } \underline{p}$$

where n = 0 to 5.

Figure IX

This series of oligosaccharides has since been isolated from many other xylans. Xylans which have not been examined in such great detail have been assumed to possess a similar basal structure from a comparison of optical rotation of the polysaccharides and their rates of hydrolysis.

As can be seen from figure VIII, xylans show a characteristic difference from cellulose as they frequently contain sugar residues other than those in the main chain. Acidic xylans contain residues of D-glucuronic acid or its 4-methyl ether. The high stability to acid hydrolysis of glycosiduronic acid linkage enables aldobiouronic acids to be easily

isolated as partial hydrolysis products from either the polysaccharide or its permethylated derivative. The structure of these hydrolysis products have been established by experiments similar to those previously described. The 1, 2 linkage, as found in the beechwood xylan (58) is by far the most common, although 1, 3 linked aldobiouronic acids are found in Monterey pine xylan hydrolysates (59) and 1, 4 linked aldobiouronic acids from the hydrolysates of corn cob xylan (60) have been reported. There is, as yet, no knowledge of the importance of these units in the structure of the xylans.

Certain similarities are apparent between xylans which have been isolated from plants of the same botanical order. All wood xylans possess 4-O-methyl -D-glucuronic acid residues but the percentage of those varies between soft woods, which have a greater proportion, and hard woods. At the same time, several soft wood xylans (61, 62, 63) also possess L-arabinofuranose side chains. Conversely, xylans from the Gramineae viftually all contain arabinose in side chains while some also possess uronic acid residues.

Xylans from the Leguminosae have not been extensively examined. The seed kernel xylan from

Tamarind has been investigated by Savur (64). The sole neutral sugar present on hydrolysis was xylose and the structural evidence for a 1, 4 linked polysaccharide is based on isolation and characterisation of 2, 3, 4 tri -, 2, 3 di- and 2 - O-methyl -D-xylose from hydrolysis of the methylated polysaccharide. The polysaccharide had a low uronic acid content (3.44%) but no structural positioning of the unit or the exact form of the acidic sugar is disclosed.

Another xylan from a legume is that isolated from lucerne (alfalfa) stems (65). The uronic acid content is appreciably higher (11%) than those from soybean hull xylan or Tamarind kernel xylan and the acidic sugar present is 4-O-methyl -D-glucuronic acid. The basal feature of the polysaccharide being that formed by 1, 4 linked β-D-xylopyranose units has been identified by methylation analysis and partial acid hydrolysis.

MANNAN

The "mannan" obtained from the alkaline borate extraction was only present in the hulls to a very small extent. Consequently, the structural investigations were limited to small-scale experiments with the results largely dependent on chromatographic techniques for the separation of the sugars and their derivatives.

Three oligosaccharides were obtained from deacetylation of the acetolysis products from the acetylated polysaccharide. They were tentatively identified by paper chromatographic and electrophoretic comparisons with the authentic sugars and by hydrolysis to the component sugars as 4 - O -β- D- mannopyranosyl- D-mannose, the polymer homologue, mannotriose, and 4-O-β-D-glucopyranosyl -D-glucose (cellubiose).

The methylated "mannan" gave cleavage products which were identified by paper chromatographic comparisons with authentic samples of the free sugars and gas-liquid chromatography of the derived methyl glycosides as 2, 3, 4, 6 tetra -, 2, 3, 6 tri - and 2, 3 di -O- methylmannose, 2, 3, 4, 6 tetra -O- methylgalactose and 2, 3, 6 tri -O-methylglucose. The major cleavage product, 2, 3, 6 tri -O- methyl -D-mannose, was avialable

in sufficient quantity to furnish a crystalline derivative. From a consideration of the results obtained from the methylation and partial acetolysis experiments, it is clear that the outstanding feature of this polysaccharide is the presence of long chains of 1, 4 linked β- D- mannopyranose units which are also terminated at the non-reducing end by mannose residues.

The only methylated galactose found in the hydrolysate of the methylated polysaccharide was 2, 3, 4, 6 tetra -O- methylgalactose. This sugar can only arise from non-reducing end units of a heteropolysaccharide. As 2, 3 di -O- methylmannose was also isolated, a small number of branch points are present in the main chain at C-6 of certain mannose residues. The resulting side chains at these points may arise from single unit galactose residues but this cannot be confirmed on the present results as no 6-O-galactopyranosylmannose was isolated from the acetolysis experiment.

The role of glucose is most easily explained in terms of a trace of a cellulose contaminant which would account for the detection of 2, 3, 6 tri -O- methyl - glucose on hydrolysis of the methylated

polysaccharide and also of cellobiose from the partial acetolysis.

The experimental evidence available suggests that this polysaccharide is a galactomannan of the structure in figure X which contains a small amount of cellulose as a contaminant.

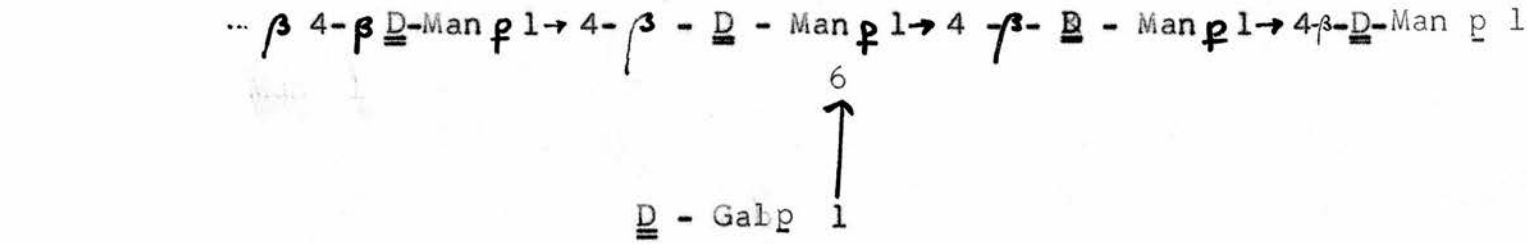


Figure X

This structure shows that the polysaccharide is related to the galactomannans isolated from soybean hulls with water (24). These three galactomannan fractions show that a spectrum of galactomannans exist in which the ratio of galactose : mannose residues vary from at least 1 : 1 . 4 to 1 : 12. The galactomannan isolated by alkaline borate extraction is the extreme end of the spectrum. The difficulty of extraction is probably due to the lack of side chains on the polysaccharide and in this respect may be compared with the mannans from vegetable ivory (66) which are also very resistant to normal extraction procedures.

The galactomannan from Locust bean gum, which has a similar structure to the soybean galactomannans can also be fractionated by dissolution in water into species with varying galactose : mannose ratios on hydrolysis (67).

CELLULOSIC POLYSACCHARIDES.

The main polysaccharide isolated from the alkaline borate extract was characterised as cellulose by the formation of cellobiose and cellotriose as the sole oligosaccharides of degradation. Cellulose itself is not generally soluble in the extractant used so the native polymer must have suffered a degree of depolymerisation itself during the total extraction sequence and this extracted cellulose must have a low molecular weight.

Similarly, the residue left over from the extraction sequence also gave large yields of cellobiose and cellotriose on partial acetolysis. These oligosaccharides are accepted as a chemical test for cellulose, giving proof not only of the 1, 4 linkage but also of the β-glycosidic configuration.

In addition, small amounts of 4 - O -β- D -manno-pyranosyl -D-mannose and the polymer homologous trisaccharide were also isolated suggesting that a small amount of residual "mannan" was also present in this residue.

ACIDIC POLYSACCHARIDES FROM SOYBEANS

Soybean Meal

The acidic polysaccharide complex from soybean meal has been subjected to partial acid hydrolyses involving 0.5- and 1.0N sulphuric acid (68). The neutral oligosaccharides which were isolated and characterised were those represented by the series XI.

$$\beta\text{-D-Gal p 1}\rightarrow[4\beta\text{-D-Gal p1}\rightarrow]_n 4\ \text{D-Gal} \qquad \text{XI}$$

where n=0 to 6.

The following acidic oligosaccharides were also isolated and characterised.

$$\alpha\text{-D-Gal pAl}\rightarrow 2\ \text{L-Rha} \qquad \text{XII}$$

$$\alpha\text{-D-GalpAl}\rightarrow 2\beta\text{-L-Rhap1}\rightarrow 4\alpha\text{-D-GalpAl}\rightarrow 2\ \text{L-Rha} \qquad \text{XIII}$$

$$\alpha\text{-D-GalpAl}\rightarrow 4\ \text{D-GalpA} \qquad \text{XIV}$$

$$\alpha\text{-D-GalpAl}\rightarrow 4\alpha\text{-D-GalpAl}\rightarrow 4\ \text{D-GalpA} \qquad \text{XV}$$

$$\beta\text{-D-GpAl}\rightarrow 4\ \text{D- Gal} \qquad \text{XVI}$$

$$\beta\text{-D-GpAl}\rightarrow 6\ \text{D- Gal} \qquad \text{XVII}$$

$$\beta\text{-D-GpAl}\rightarrow 4\ \text{L- Fuc} \qquad \text{XVIII}$$

In the present investigation a further sample of the same polysaccharide fraction from soybean meal has been subjected to graded acetolysis, when a mixture of mono-and oligosaccharides was obtained on deacetylation of the acetolysis products. This mixture was separated into neutral and acidic components on DEAE-Sephadex. The

neutral sugars were themselves fractionated on a charcoal-Celite column and, where necessary, on filter sheets.

As well as giving a mixture of galactose, arabinose, xylose and fucose, two methylated sugars were obtained which were identified as 2-O-methylxylose and 2-O-methylfucose by paper chromatography, and gas-liquid chromatography of the derived methyl glycosides.

Among the neutral oligosaccharides isolated from the acetolysis experiment were the oligosaccharides galactobiose to galactoheptaose of the series XI (n=0 to 5). The first member of this series crystallised from ethanol-water and was identical to an authentic sample of 4-O-β-D-galactopyranosyl -D-galactose.

The other members of the series (n=1 to 5) were initially identified by paper chromatographic examination of the partial acid hydrolysis products when the lower oligosaccharides of the series were obtained and by gas-liquid chromatographic results from the methanolysis products of themethylated oligosaccharides when peaks were obtained with the retention times of the methyl glycosides of 2,3,4,6 tetra- and 2,3,6 tri -O-methyl-galactose. The galactotriose gave a crystalline hendeca-acetate which was identical to a sample prepared from O-β-D-galactopyranosyl (1→4)-O-β-D-galactopyranosyl (1→4)-D-galactose obtained from the partial acid hydrolysis of soybean

meal polysaccharide (68). The galactotetraose and galactopentaose (n = 2 and 3) gave crystalline tetradeca- and heptadeca-acetates respectively.

Two further galactose containing oligosaccharides were obtained, each giving a mauve colour with the hydroxylamine-ferric chloride spray. Both sugars were treated with 1% ammonia when one gave a sugar similar to galactobiose and the other gave a sugar similar to galactotriose (XI n= O, 1). These sugars are evidently acetylated derivatives of galactobiose and galactotriose which had not been completely deacetylated during the working up of the acetolysis products.

An oligosaccharide was present which gave galactose and xylose on hydrolysis while its derived glycitol gave galactose and xylitol. The cleavage products from the methylated disaccharide were characterised as 2,3,4,6 tetra-O-methyl -D-galactose and 3,4 di -O-methyl -D-xylose. These results, taken with the optical rotation of the sugar (-36^{o}) suggest that the sugar is 2-O-β-D-galactopyranosyl -D-xylose (XIX).

β -D- Gal p 1→2 D-Xyl XIX

An oligosaccharide contained fucose and xylose residues while its derived glycitol gave fucose and xylitol on hydrolysis. The methyanolysis products from the methylated disaccharide were examined by gas-liquid chromatography when peaks were

obtained corresponding to the methyl glycosides of 2,3,4 tri -O-methyl fucose and 3,4 di -O- methylxylose. The sugar had an optical rotation of -59° suggesting that it was 2-O-α-L-fucopyranosyl -D-xylose XX

α -L- Fuc p 1→2 D - Xyl XX

Two oligosaccharides were obtained which contained only arabinose residues. One appeared to be a disaccharide and the other a trisaccharide while both contained arabinofuranose residues as non-reducing terminal units. On the available evidence, however, their structures could not be assessed.

Two further oligosaccharides were obtained, one containing arabinose, rhamnose and xylose residues and the other galactose, arabinose and xylose but due to the small amounts present, they were not examined further.

Four acidic oligosaccharides were obtained from the acetolysis of soybean meal polysaccharide complex. The first was identified as 2-O- (α-D-galactopyranosyluronic acid) -L-rhamnose (XII) by formation of its crystalline methyl glycoside pentamethyl ether dihydrate.

α -D-Gal pA1→2 L-Rha XII

The second acidic oligosaccharide contained galacturonic acid and rhamnose residues in the ratio 1 : 1.9(40,42) and

gave 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose and rhamnose on partial hydrolysis. The derived glycitol contained the same residues in the ratio 1 : 1 and gave the same aldobiouronic acid and rhamnitol on partial acid hydrolysis. The sugar gave a yellow spot with the periodate - Schiff spray (33) while the glycitol gave a blue one. These results suggest that the reducing sugar is substituted at C-2. The methanolysis products from the methylated trisaccharide contained the methyl glycosides of 3,4 di -O-methylrhamnose and 2,3,4 tri -O-methylgalacturonic acid. The structure is probably that of XXI if the galacturonic acid residue is assumed to have the α-configuration as in the aldobiouronic acid XII.

α-D-Gal pAl→2L-Rhap1→2 L-Rha XXI

The third acidic oligosaccharide contained galacturonic acid and rhamnose residues in the ratio 1 : 1 while its glycitol gave the same sugars in the ratio 2 : 1. The sugar gave 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose on partial hydrolysis while the glycitol gave the same aldobiouronic acid and the glycitol 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnitol. The sugar gave a yellow colour with the periodate-Schiff spray (33) while a blue one was obtained from the glycitol. These colours would be expected if the reducing rhamnose residue was substituted at C-2. The methylated

tetrasaccharide was methanolysed and the products were examined by gas-liquid chromatography. Peaks were obtained with the retention times of the methyl glycodies of 3,4 di -O-methylrhamnose, 2,3 di- and 2,3,4 tri -O-methylgalacturonic acid, suggesting that the structure was XIII

α-D- Gal pAl→2β-L-Rhap 1→4α-D- GalpAl→2 L-Rha XIII

The last acidic oligosaccharide contained galacturonic acid and rhamnose in the ratio of 1.1 : 1 while its glycitol contained the same residues in the ratio 1.9 : 1. The sugar gave spots similar to digalacturonosylrhamnose and rhamnose on partial hydrolysis while the glycitol gave the same sugar and rhamnitol. Yellow and blue spots were obtained respectively from the sugar and the glycitol with the periodate-Schiff spray (33) again suggesting the reducing rhamnose residue was 2- substituted. Gas-liquid chromatographic examination of the methanolysis products from the methylated tetrasaccharide gave peaks with the retention times of the methyl glycosides of 3,4 di -O- methylrhamnose, 2,3 di- and 2,3 4 tri -O-methyl-galacturonic acid. These results suggest that the sugar has structure XXII.

D- Gal pAl→4 D-GalpAl→2L - Rha p1→2L-Rha XXII

Soybean Hulls.

As may be recalled, various acidic polysaccharide fractions (I, II, IV and V) have been isolated from soybean hulls. I and

II were extracted with cold and hot water respectively while IV was extracted with ethylenediaminetetra-acetic acid and V with sodium hydroxide. I and II both appeared to be homogeneous on precipitation but small amounts of galactomannan were shown to be present by DEAE-cellulose chromatography on both polysaccharides. Acidic polysaccharide IV gave two peaks on DEAE-cellulose chromatography but this result is explained in variations in the degree of esterification of the polysaccharide. Acidic polysaccharide V appeared to be homogeneous. Each of these polysaccharides was partiallly hydrolysed with mineral acid and subjected to partial acetolysis on a small scale. The major oligosaccharides produced were similar in each case suggesting that similarities also existed between the original polysaccharides. As only limited amounts of each polysaccharide were available it was not possible to carry out an extensive examination of each one. Acidic polysaccharides I and II were combined as they appeared to be virtually identical. Examination of the cleavage products from hydrolysis of those pdysaccharides was limited to the acidic oligosaccharides as the presence of galactomannan contaminant would lead to additional neutral oligosaccharides. In this connection it may be noted that the presence of mannose residues in the

hydrolysates of these polysaccharides does not provide a satisfactory criterion of their heterogeneity since later a mannose containing aldobiouronic acid was isolated from the partial acid hydrolysate. Acidic polysaccharides IV and V were separately subjected to graded acetolysis to examine both neutral and acidic fragments.

Combined acidic polysaccharides I and II from soybean hulls were hydrolysed with N-sulphuric acid. A degraded polysaccharide separated out during hydrolysis and a further quantity was recovered by precipitation with acetone before the mixture of sugars was separated into neutral and acidic components on DEAE-Sephadex. The acidic sugars were themselves separated on DEAE-Sephadex and, where necessary, on filter sheets.

The major oligosaccharide component was characterised as 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose (XII) by formation of its crystalline methyl glycoside pentamethyl ether dihydrate.

An oligosaccharide was obtained containing galacturonic acid and rhamnose residues in the ratio 1.1 : 1 while the derived glycitol contained the same residues in the ratio 2.1 : 1. Partial hydrolysis of the tetrasaccharide gave mainly 2-O-(α-D-galactopyranosyluronic acid) -L-rhamnose while the glycitol gave the same aldobiouronic acid and a sugar alcohol with the

chromatographic mobility of 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnitol. Gas liquid chromatography of the methanolysis products from the methylated tetrasaccharide and methylated glycitol gave peaks with the retention times of 3,4 di -O-methylrhamnose, 2,3 di- and 2,3,4 tri -O-methyl galacturonic acid, and the same sugars and 1,3,4,5 tetra -O-methylrhamnitol respectively. Methanolysis of the reduced methylated tetrasaccharide gave components with the retention times of the methyl glycosides of 3,4 di -O-methylrhamnose and 2,3,4 tri -O-methylgalactose. Although not detected by gas-liquid chromatography, hydrolysis of this sample indicated the presence of 2,3 di -O-methylgalactose by paper chromatography. This sugar is evidently a linear molecule with the structure XIII. On assuming that both galacturonic acid residues have an α-configuration, the molecular rotation of the sugar (+635), compared with the sum of the molecular rotations of two galacturonosylrhamnose residues (+585), suggests that the non-reducing rhamnose residue has a β-configuration.

α-D- GalpAl → 2β-L-Rhapl → 4α-D-GalpAl → 2L-Rha XIII

A further oligosaccharide containing galacturonic acid and rhamnose residues in the ratio of 1.9 : 1 was isolated. The derived glycitol was devoid of rhamnose residues. The sugar was resistant to mild acid hydrolysis but more vigorous

hydrolysis gave, as well as galacturonic acid and rhamnose, a sugar with the mobility of 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose. Reduction of the derived methyl ester methyl glycosides followed by methylation and methanolysis gave peaks on gas-liquid chromatography with the retention times of the methyl glycosides of 3,4 di -O-methylrhamnose, 2,3, 6 tri- and 2,3,4,6 tetra -O-methylgalactose. These observations suggest that the structure of the trisaccharide is that of XXIII. Assuming the configuration of the non-terminal galacturonic acid residue to be α, the molecular rotation of the sugar (+413) does not clearly indicate the configuration of the glycosidic linkage of the terminal galacturonic acid residue.

D- GalpAl→ 4α-D-GalpAl→ 2 L-Rha XXIII

Two acidic oligosaccharides were obtained which gave only galacturonic acid on hydrolysis and were chromatographically and ionophoretically indistinguishable from 4-O-(α-D-galacto-pyranosyluronic acid) -D- galactopyranosyluronic acid (XIV) and the polymer homologue trigalacturonic acid (XV). Both sugars furnished calcium salts with optical rotations similar to the calcium salts of di- and trigalacturonic acid respectively.

α -D- GalpAl→ 4 D- GalpA XIV

α -D-GalpAl→ 4α-D- GalpAl→ 4 D-Gal pA XV

Four acidic oligosaccharides containing glucuronic acid as one of the constituent sugars were isolated in small yields. They were all characterised, as far as possible, by small-scale experiments. These involved paper chromatography of the hydrolysis products from the aldobiouronic acid, its derived glycitol and the neutral sugar obtained by reduction of the derived methyl ester methyl glycosides of the aldobiouronic acid, and gas-liquid chromatography of the methanolysis products from the methylated aldobiouronic acid or a derivative of it. The structures of the sugars are given below, the glycosidic configurations being suggested by the optical rotations of the sugars.

β-D-G pAl→4 D Gal XVI

β-D-G pAl→6 D-Gal XVII

β-D-G pAl→4 L-Fuc XVIII

β-D-G pAl→2 D-Man XXIV

Acidic Polysaccharide IV from soybean hulls was subjected to graded acetolysis and the mixture of sugars was separated as described for soybean meal polysaccharide complex. A mixture of 2-O-methylxylose and 2-O-methylfucose, identified by paper chromatography, was obtained along with five neutral oligosaccharides. All these, except the first, which crystallised from ethanol - water and was identical to 4-O-β-D-galactopyranosyl -D-galactose (XI, n=0), were characterised

by small scale experiments. These involved paper chromatographic examination of the partial hydrolysis products from the sugar and its derived glycitol and gas-liquid chromatography of the methanolysis products from the methylated sugars. These sugars were:-

α-L-Fucp 1→2 D Xyl	XX
β-D-Galp 1→2 D Xyl	XIX
Galactotriose	XI n = 1
Galactotetraose	XI n = 2

Acidic polysaccharide V from soybean hulls was subjected to graded acetolysis as described for acidic polysaccharide IV and gave a mixture of four neutral oligosaccharides which were characterised by the same small-scale experiments outlined for acidic polysaccharide IV. These oligosaccharides were

β-D- Gal p 1→4 D- Gal	XI n = 0
β-D- Gal p 1→2 D- Xyl	XIX
α-L- Fuc p 1→2 D- Xyl	XX
β-D- Gal p 1→4 β-D-Gal p 1→4 D- Gal	XI n = 1

Three acidic oligosaccharides were also isolated from Acidic polysaccharide IV from soybean hulls. The first was identified as 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose by conversion into its crystalline methyl glycoside pentamethyl ether dihydrate.

The second acidic oligosaccharide was chromatographically similar to oligosaccharide XXI from soybean meal polysaccharide complex and gave similar results to the same experiments. It was further characterised as XXI by examination of the methanolysis products from the methylated glycitol when 1,3,4,5 tetra -O-methylrhamnitol and the methyl glycosides of 3,4 di -O-methylrhamnose and 2,3,4 tri -O-methylgalacturonic acid were identified by gas-liquid chromatography.

α-D- Gal pAl→2 L-Rha p 1→2 L - Rha XXI

The last acidic oligosaccharide from acidic polysaccharide IV was chromatographically identical to oligosaccharide XIII from soybean meal polysaccharide complex and gave the same results to the same experiments. The methylated tetrasaccharide glycitol gave, on methanolysis, components with the retention times of 1, 3,4,5 tetra-O-methylrhamnitol and the methyl glycosides of 3,4 di -O-methylrhamnose, 2,3 di- and 2,3,4 tri-O-methylgalacturonic acid. The sugar is therefore

α-D-Gal p Al→2β-L-Rhap1→4α-D- GalpAl→2 L- Rha XIII

The only acidic oligosaccharide isolated in sufficient quantity for identification from the acetolysis of acidic polysaccharide V was identified as 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose (XII) by gas-liquid chromatographic examination of the methanolysis products from the methylated aldobiouronic acid.

The oligosaccharides obtained from the breakdown of acidic polysaccharides from soybeans are summarised in the tables on the following pages, which include previous (68) and present work.

NEUTRAL OLIGOSACCHARIDES

Oligosaccharide	Hulls IV Acetolysis (A)	Hulls V Acetolysis (A)	Meal: Acid Hydrolysis (B)	Meal: Partial Acetolysis (A)
Gal 1β→[4 Gal 1β→]$_n$4 Gal	+ n=0, 1,2	+ n=0, 1	+ n=0 to 6	+ n=0 to 5
Fuc 1α→2 Xyl	+	+	-	+
Gal 1β→2 Xyl	+	+	-	+

A = present work B = previous work

Acidic Oligosaccharides.

Oligosaccharide	Hulls I + II Acid Hydrolysis	Hulls IV Acetolysis	Hulls V Acetolysis	Meal:acid Hydrolysis	Meal: Acetolysis
Gal A 1⇒2 Rha	A +	A +	A +	B +	A +
Gal A 1⇒2 Rha 1⇒4 Gal A 1⇒2 Rha	+	+	-	+	+
Gal A 1⇒2 Rha 1→2 Rha	-	+	-	-	+
Gal A 1→4 Gal A 1⇒2 Rha	+	-	-	-	
Gal A 1→4 Gal A 1⇒2 Rha 1→2 Rha	-	-	-	-	+
Gal A 1⇒4 Gal A	+	-	-	+	
Gal A 1⇒4 Gal A 1⇒4 Gal A	+	-	-	+	
G A 1⇒6 Gal	+	-	-	+	
G A 1⇒4 Gal	+	-	-	+	
G A 1⇒4 Fuc	+	-	-	+	
G A 1⇒2 Man	+	-	-	-	

A = present investigation B = previous investigation.

Methylation Results.

Acidic polysaccharides IV and V from soybean hulls were separately converted to their methyl esters and methylated firstly in a mixture of dimethylsulphoxide and N, N dimethylformamide with methyl sulphate and crushed barium hydroxide and finally with methyl iodide and silver oxide in N, N dimethylformamide. After fractional dissolution in mixtures of chloroform and light petroleum, fully methylated samples were obtained. The methanolysis products from these samples were analysed by gas liquid chromatography, when peaks were obtained corresponding in retention times to the methyl glycosides of following sugars:-

2,3,4,6 tetra -O- methylgalactose

2,3,4 tri -O- methylxylose

2,3,4 tri -O-methylfucose

3,4 di -O-methylxylose

2,3,6 tri -O-methylgalactose

2,3,5 tri -O-methylarabinose

2,3 di -O-methylarabinose(?)

2,3 di -O-methylxylose

3,4 di -O-methylrhamnose

3 -O-methylrhamnose

2,3 di -O-methylgalacturonic acid

2,3,4 tri -O-methylgalacturonic acid

The syrup of methyl glycosides from both polysaccharides was reduced with lithium aluminium hydride in tetrahydrofuran and hydrolysed. The sugars observed on paper chromatography and ionophoresis other than those already mentioned were

2,3,4 tri -O- methylgalactose,

2,3 di -O-methylgalactose

2-O- methylgalactose

3-O- methylgalactose

galactose.

All the above mentioned sugars were obtained as cleavage products from methylated acidic polysaccharides IV and V from soybean hulls. It is assumed that the five derivatives of galactose given immediately above are derived from the reduction of galacturonic acid residues present in the original polysaccharide.

Evaluation of the Structures of Soybean Acidic Polysaccharides.

The isolation of a number of galacturonic acid and rhamnose containing oligosaccharides from partial depolymerisation of the various soybean acidic polysaccharides suggests that these sugars are constituents of the main chain of these polysaccharides. The sequence of these sugar residues can also be partially determined from examination of these oligosaccharides. Galacturonic acid residues can occupy

adjacent sites where up to at least three galacturonic acid residues occur together. Contiguous rhamnose residues can also occur but no more than two of these have been found together. If A represents galacturonic acid and B, rhamnose, a typical basal chain is given in XXIII.

···A-B-A-A-B-A-B-B-A-A-A-B-B-A-B-A···

XXIII

In all the oligosaccharides isolated in which galacturonic acid was found not occupying a non-reducing terminal site, it was position C-4 which was substituted. Under similar circumstances, position C-2 on rhamnose residues was substituted as is shown in XXIV.

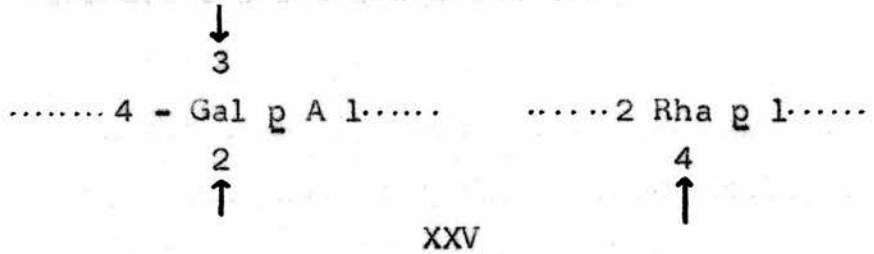
D-GalpA 1→4DGalpA 1→2 LRha p1→2 L-Rhap1→4 D Gal pA

XXIV

Several of the galacturonic acid residues have an α-glycosidic configuration but it is also possible that some may have a β-configuration. No real evidence is available regarding the glycosidic configuration of rhamnose residues but some are tentatively assigned a β-configuration.

The galacturonic acid and rhamnose containing oligosaccharides do not give any information about branching of the main chain. The methylation results from soybean hull polysaccharides IV and V show the presence of 2- and 3-O-methylgalactose, galactose and

3-O-methylrhamnose from the carboxyl reduced methylated polysaccharide. If the galactose residues are assumed to arise from galacturonic acid residues in the original polysaccharides, branch points occur at C-2 and/or C-3 of certain galacturonic acid residue and at C-4 of certain rhamnose residues as illustrated in XXV



XXV

The nature of these side chains is very complex and it is as yet impossible to say where they are attached. The side chains which have been found as oligosaccharide fragments and whose structure have been further confirmed by the methylation evidence are:-

β-D-Gal p 1→[4 β-D-Gal p 1→]$_n$4 D Gal XI

β-D-Gal p 1→2 D - Xyl XIX

∝-L- Fucp1→ 2 D - Xyl XX

No definite interpretation can be placed on the isolation of the four aldobiouronic acids from acidic polysaccharides I and II from the hulls, namely,

β-D-G pA 1→4 D-Gal XVI

β-D-G pA 1→6 D-Gal XVII

β-D-Gp A 1→4 L-Fuc XVIII

β-D-Gp A 1→2 D-Man XXIV

It will be recalled that XVI, XVII and XVIII were also isolated from partial acid hydrolysis of soybean meal polysaccharide complex (68). The last of these aldobiouronic acids, XXIV may be peculiar to acidic polysaccharides I and II from the hulls as no mannose has been detected in hydrolysates of acidic polysaccharides IV and V from the hulls.

It may be suggested that some of the side chains formed by oligosaccharide XX may be further substituted by glucuronic acid residues to give side-chains of the form:-

β-D- G pA 1→4α-L-Fucp 1→2 D Xyl

The other two acidic sugars XVI and XVII may similarly arise from further substitution of the side chains XI and XIX.

2,3,4 Tri -O-methylxylose was observed in the cleavage products of the methylated polysaccharides IV and V but it is not yet possible to say if it arises from xylopyranose residues as single unit side chains.

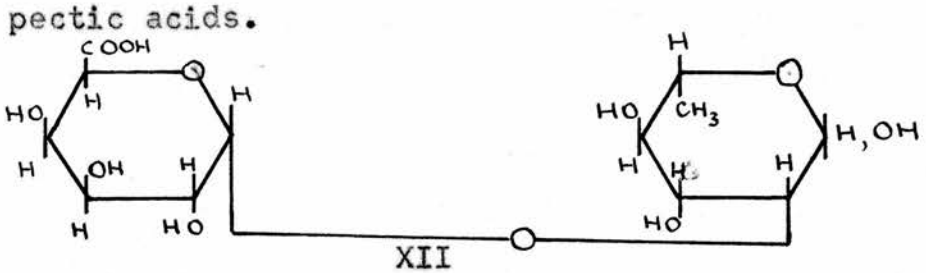
The acidic polysaccharides from soybeans have still many structural features which are not completely revealed by these results, the most notable being the exact points of linkage of side units, more detail about the fine structure of those side units and the location of the arabinose residues.

Comparison of the Structural Features of Soybean Acidic Polysaccharides with other Acidic Polysaccharides from Land Plants.

The classical idea that the gel forming material found in the cell-wall of most land plants consisted of three homopolysaccharides, a galacturonan, a galactan and an araban must be modified from our present knowledge of pectic substances. Although authenticated examples of each of these types of polysaccharide are known, there is much evidence that many galacturonans contain neutral sugar residues as integral parts of their structure.

Conventional precipitation techniques have resulted in apparently homogeneous pectic acids being obtained from several fruit sources (69), which still contained galactose, arabinose, xylose and rhamnose residues. Sisal pectic acid also gives a fraction which is resistant to further precipitation (70). This fraction gave galactose, arabinose and rhamnose on hydrolysis as well as two methylated sugars, identified as 2-O- methylxylose and 2-O-methylfucose. The only sugar, however, which has been found directly linked to galacturonic acid is rhamnose in the aldobiouronic acid, 2-O-(α-D-galacto-pyranosyluronic acid) -L- rhamnose (XII) obtained from partial

acid hydrolysates of grape juice (71) and lucerne (72) pectic acids.



XII

The advent of DEAE-cellulose has proved useful in looking for neutral sugar residues in pectic acids. Two groups of workers (35, 73) have shown that pectic materials can be fractionated into neutral and acidic components on this ion-exchange medium. In all cases, the recovered polysaccharide contained galactose and arabinose residues as well as galacturonic acid. The eluted acidic polysaccharide, when free from neutral material, still was not eluted as a sharp band due to variations in the degree of esterification of the polysaccharide.

Apple fruit pectic substances have recently been fractionated into an acidic component and an araban-galactan complex (74). The acidic component was homogeneous on ultracentrifugation, free-boundary and zone electrophoresis, but still gave on hydrolysis galactose, arabinose, xylose, rhamnose, fucose and traces of 2-O- methylxylose and 2-O-methylfucose as well as galacturonic acid.

Battacharjee and Timell (75) have isolated, after removal of other contaminating polysaccharides, an acidic polysaccharide from Amabilis Fir which appeared to be homogeneous on precipitation. Electrophoresis showed this was not so, and preparative ultracentrifugation has given a homogalacturonan and a pectic acid. Both polysaccharides are based on 1, 4 linked chains of α-D-galactopyranosyl-uronic acid residues but the pectic acid also contains residues of galactose, arabinose and rhamnose.

Acidic polysaccharide III from soybean hulls (27) which was extracted with 0.5% ammonium oxalate solution was homogeneous on free-boundary electrophoresis and DEAE-cellulose chromatography. The major oligosaccharides produced on partial acid hydrolysis were 2-O-(α-D-galactopyranosyluronic acid) -L-rhamnose, and 1, 4 linked oligomers of galacturonic acid. The methanolysis products from the methylated polysaccharide were qualitatively similar to those from acidic polysaccharides IV and V from soybean hulls.

The polysaccharide complex from soybean meal has been fractionated into an acidic polysaccharide and a neutral component, which is either an arabinogalactan or an araban-galactan mixture (68, 76.) As the neutral component is eluted as a single peak on DEAE cellulose chromatography in the borate form, it is probably the former, as pectic arabans (77) are strongly adsorbed under these conditions. The acidic component is eluted

as two bands from DEAE-cellulose but there does not seem to be any significant difference between them.

The major oligosaccharides obtained from the partial acid hydrolysis of soybean meal acidic polysaccharide complex are shown again below.

β-D-Gal p 1→[4β-D- Galp 1→]$_n$4 D- Gal XI

α-D- Gal p A 1→2 L-Rha XII

α-D-Gal p A1→2β-L-Rhap1→4α-D- GalpA1→2 L-Rha XIII

α-D-GalpA 1→4 D-Gal pA XIV

α-D-GalpA1→4α-D-GalpA 1→4 D-Gal pA XV

β-D- G p A 1→4 D-Gal XVI

β-D- G p A 1→6 D-Gal XVII

β-D- G p A 1→4 L-Fuc XVIII

Further evidence for the fact that unbranched chains of 1,4 linked galactose residues as in XI are present has come from preliminary methylation evidence when large amounts of 2,3,6 tri -O-methylgalactose were detected. From this same examination, it would appear that glucuronic acid residues occupied non-reducing terminal sites.

Citrus pectic acid, when free from any contaminating neutral galactan, has also given the sugars galactobiose and galactotriose of the series XI (n=0,1) on partial depolymerisation (78) showing that chains of 1, 4 linked galactose residues are also integral parts of citrus pectic acid.

The acidic polysaccharides from soybeans have shown certain features which have also been noted in Tragacanthic acid, the acidic component from gum tragacanth. Partial acetolysis of gum tragacanth (79) has yielded the oligosaccharides:-

β -D- Gal p 1→2 D Xyl XIX

α -L- Fuc p 1→2 D Xyl XX

while more recently, partial acid hydrolysis has yielded the aldobiouronic acids (80).

α -D-Gal p A 1→2 L-Rha

β -D-GpA 1→4 L-Fuc

α -D-GpA 1→4 D-Gal

A comparison can be made between the partial depolymerisation products of soybean acidic polysaccharides, citrus pectic acid (which is a more typical example of a pectic acid) and tragacanthic acid and these products are shown in the following table:-

TABLE

Acidic Polysaccharides from Soybeans	Citrus Pectin	Tragacanthic Acid
Gal A 1⇒4 Gal A GalAl⇒4 GalAl⇒4 Gal A Gal A 1⇒2 Rha + others	Gal A 1⇒4 Gal A GalAl⇒4 GalAl⇒4 GalA Gal A 1⇒2 Rha	Gal A 1⇒4 Gal A GalAl⇒4GalAl⇒4Gal A Gal A 1⇒2 Rha
Gal 1β⇒4 Gal Gal 1β⇒4 Gal 1β⇒4 Gal etc.	Gal 1β⇒4 Gal Gal 1β⇒4 Gal 1β⇒4 Gal	
Fuc 1⇒2 Xyl Gal 1β⇒2 Xyl		Fuc 1⇒2 Xyl Gal 1β⇒2 Xyl
GAl β⇒4 Fuc GAl β⇒4 Gal GAl β⇒6 Gal		GAl β⇒4 Fuc GAl ⇒4 Gal

Tragacanthic acid has very few rhamnose residues compared to Galacturonic acid and in these acidic polysaccharides we may have a spectrum of polysaccharides based on a galacturonic acid-rhamnose backbone with Tragacanthic acid on one hand and the gums of the Khaya and Sterculia families on the other as these latter gums have a galacturonic acid: rhamnose ratio which is near unity.

In tragacanthic acid, it is known that the side chains are attached to C-3 of galacturonic acid residues (79).

Glucuronic acid residues are more commonly found as a non-reducing terminal unit in plant gums with a galactan framework. The aldobiouronic acid, 6-O-(β-D-Glucopyranosyluronic acid) -D-galactose is found in hydrolysates of Acacia gums (81). The same oligosaccharide has been isolated from partial acid hydrolysates of gums of the Anogeissus genus (82, 83) as has 2-O-(β-D-glucopyranosyluronic acid) -D-mannose. In the case of leiocarpan A, one of the polysaccharide components of Anogeissus leiocerpa (syn. A. schimperi) gum, the latter aldobiouronic acid has now been shown to comprise the repeating unit of the main chain (84).

EXPERIMENTAL

EXTRACTION OF SOYBEAN HULLS.

Delignification

Soybean hulls (1 Kg.), which had previously been extracted with cold and hot (60°) water and 0.5% ammonium oxalate solution were delignified by the procedure of Timell and Jahn (49). Chlorine was bubbled through a suspension of the hulls in water at 5°for 5 min., the solution was filtered and the residue was extracted with 3% ethanolic ethanolamine solution in a Soxhlet extractor overnight. This process was repeated until no further colour was observed in the extractant (4 times.)

Extraction with EDTA.

Delignified hulls were heated at 95° with a 2% solution of ethylenediaminetetra-acetic acid disodium salt (4 x 7l.) for periods of four hours. On centrifuging hot and cooling the centrifigate, polysaccharide was precipitated by addition of ethanol (1 vol.). No polysaccharide was left in solution. The crude polysaccharide (37 g.) was dissolved in water, dialysed against running tap-water

for 3 days to remove inorganic salts and reprecipitated by addition of ethanol (1 vol.). Washing of the polysaccharide with ethanol, acetone and ether, and drying in a dessicator gave polysaccharide E(29.5 g.)

Extraction with 10% Potassium Hydroxide.

The residue from the above extraction was extracted overnight under nitrogen with 10% potassium hydroxide solution (3 x 7l.). Neutralisation of the centrifugates with acetic acid gave Hemicellulose 1 (57 g.) which was centrifuged off and dried by solvent exchange. Addition of ethanol (1 vol.) gave Hemicellulose 2 (14.2g.).

Extraction with Sodium Hydroxide containing Boric Acid.

The residue from the above extraction was further extracted with 10% sodium hydroxide containing 4% boric acid (2 x 7l.) after the procedure of Jones, Wise and Jappe (51). Hemicellulose 3 (56 g.) was recovered by neutralisation of the extract and Hemicellulose 4 (3.2g.), on addition of ethanol (1 vol.). Both polysaccharides were washed with hot 95% ethanol to remove boric acid before drying by solvent exchange.

The results of these extractions are summarised in table I.

TABLE I.

Extract	Uronic Acid	N	Ash	$[\alpha]_D$	Sugars on Hydrolysis							
	Anhydride [a] %	%	%		G	Gal	Man	Ara	Xyl	Rha	Fuc.	A.A.
E	18	1.3	10	+7	+	+++	-	+++	+++	+	tr	+
1	6	0.5	5	-60	+	+	-	+	+++	-	-	tr
2	18	0.6	6	-	+	+++	-	++	+++	+	tr	+
3	O	-	-	-	+++	-	+	-	tri	-	-	-
4	O	-	-	-	+	+	+++	-	++	-	-	-
R	O	-	-	-	+++	-	tr	-	tri	-	-	-

a - by decarboxylation tr - trace.

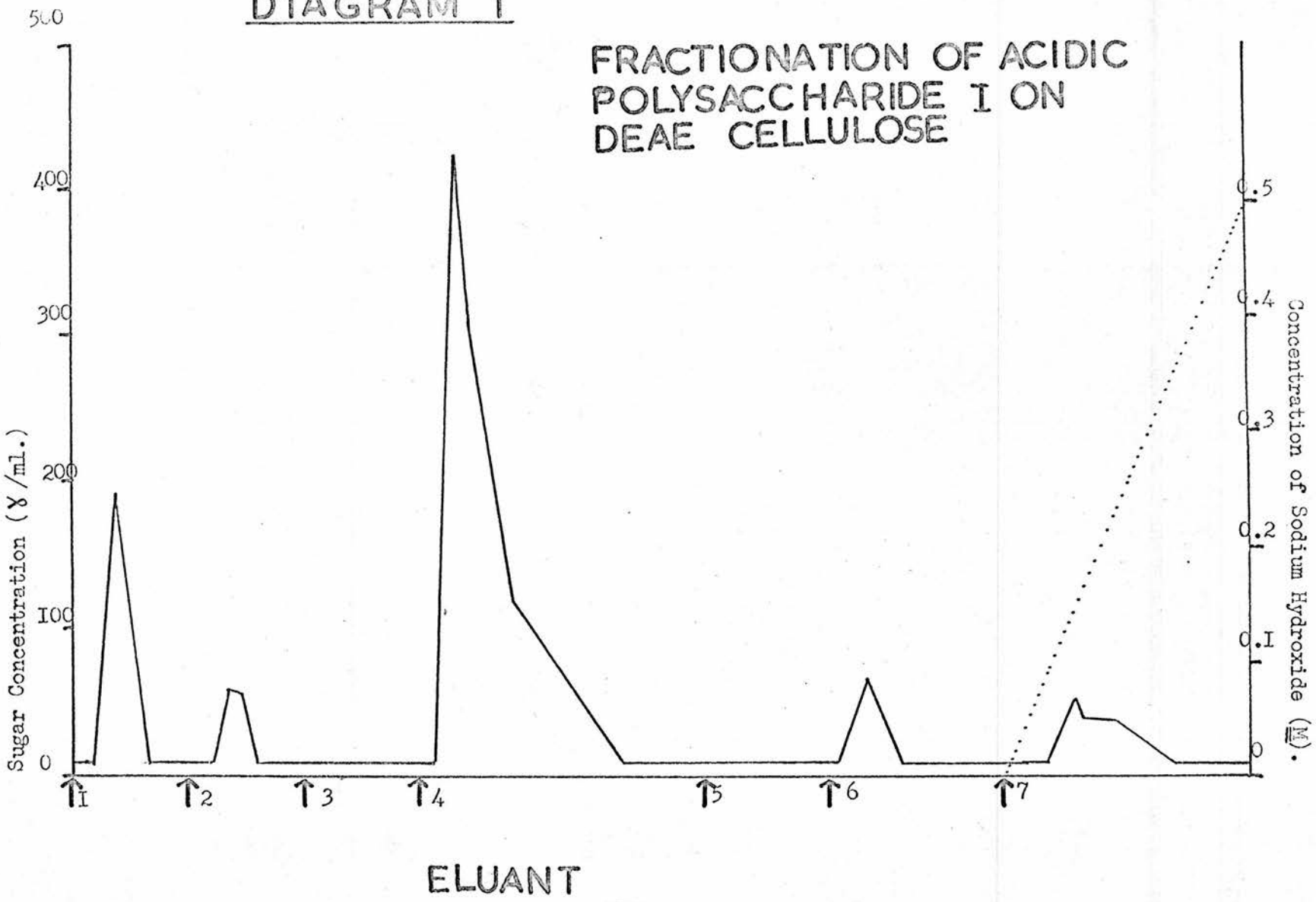
FRACTIONATION OF HULL EXTRACTS.

Cold Water Extract

Fractionation with Copper Acetate

Soybean hulls, when extracted with water at room temperature, give an extract that can be fractionated into a Galactomannan I and an Acidic Polysaccharide I with copper acetate (24). The acidic polysaccharide (11 g.) was dissolved in water (1l.) and retreated with 7% copper acetate solution (150 ml.) An acidic polysaccharide was precipitated as its copper salt while addition of ethanol (1 vol.) gave a further polysaccharide fraction. The copper salts were decomposed with ethanol containing 1% hydrogen chloride and dried to give acidic polysaccharide (5.9 g.) from the insoluble copper salt and a polysaccharide (3.6 g.) which appeared to be further galactomannan from its hydrolysis pattern, from the soluble copper salt. The acidic material was reprecipitated in the same way to give a pure Acidic Polysaccharide I (5.8 g.), $[\alpha]_D$ +140° (c 1.2) [Found: uronic anhydride (by decarboxylation), 39%], which gave on hydrolysis galacturonic acid, galactose, mannose, arabinose, xylose and traces of rhamnose, fucose and two

DIAGRAM I



I,0.05-,2,0.1-,3,0.2-,4,0.5M Phosphate 5,0.2-,6,0.5M Chloride 7- Hydroxide

methylated sugars. No further precipitate was obtained from the supernatant liquid by addition of ethanol.

Fractionation of Acidic Polysaccharide I on DEAE Cellulose.

A sample (127 mg.) of acidic polysaccharide I was chromatographed on diethylaminoethylcellulose (30 g.), generated in the phosphate form. The column was eluted successively with 0.025-, 0.05-, 0.1-, 0.2- and 0.5M sodium dihydrogen phosphate buffers at pH 6.1, 0.1, 0.2 and 0.5M sodium hydroxide. Fractions were collected (25 ml.) and analysed by the phenol-sulphuric acid method for total sugars and the carbazole method for uronic acids. A plot of polysaccharide content against eluant is given in the corresponding diagram 1. In all, five polysaccharide fractions were obtained, the major one (*ca* 70%) being eluted with 0.5M phosphate buffer and having $[\alpha]_D$ + 150° (*c* 0.34) [Found: uronic acid (by decarboxylation), 40%]. The yields and hydrolysis patterns of the fractions are given in table II

TABLE II.

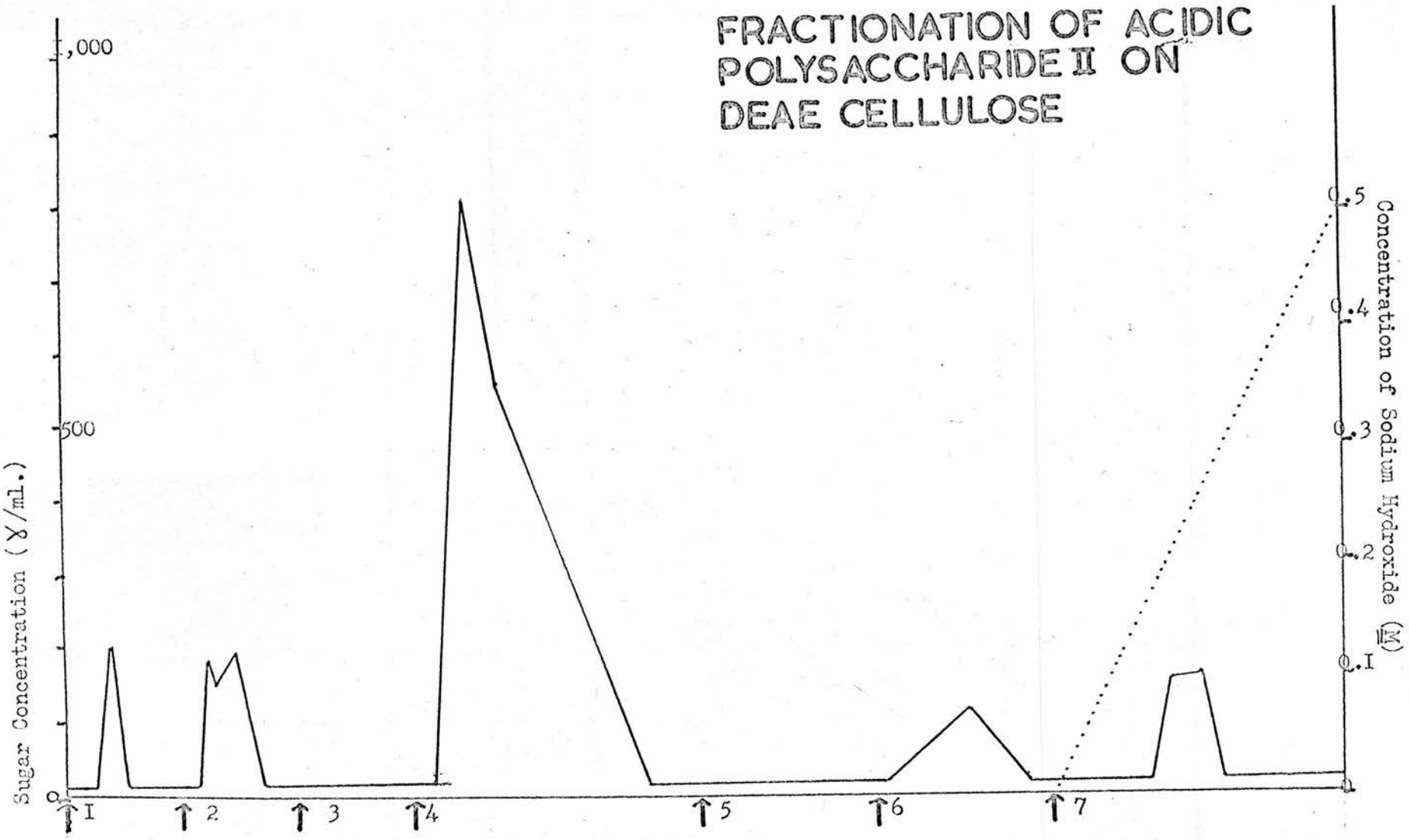
Eluant	Wt(mg.)	Sugars on Hydrolysis.							
		G	Gal	Man	Ara	Xyl	Rha	Fuc	A.A.
0.05M-PO_4^{III}	8	-	+	++	tr	++	tr	-	-
0.1M-PO_4^{III}	3	tr	+	++	+	++	+	+	++
0.5M-PO_4^{III}	68	tr	+	+	+	++	+	+	++
0.5M-Cl^{I}	10	tr	+	+	+	++	+	+	++
0.3M-OH^{I}	5	tr	+	+	+	++	+	+	++

Hot Water Extract.

Fractionation with Copper Acetate.

The hot (60°) water extract from soybean hulls also gives a Galactomannan II and an Acidic Polysaccharide II on fractionation with copper acetate (24.) The acidic polysaccharide (14 g.) was dissolved in water (1 l.) and treated with copper acetate solution in a similar way to the cold water extract. Acidic polysaccharide (5.9 g.) was recovered as its insoluble copper salt and a further yield of galactomannan from the soluble copper salt. The acidic polysaccharide was again reprecipitated in the same way to give Acidic Polysaccharide II (5.1 g.),

DIAGRAM II


FRACTIONATION OF ACIDIC POLYSACCHARIDE II ON DEAE CELLULOSE
,000
500
0
Sugar Concentration (γ/ml.)
0.5
0.4
0.3
0.2
0.1
0
Concentration of Sodium Hydroxide (M)
1
2
3
4
5
6
7
ELUANT
I, 0.05-, 2, 0.I-, 3, 0.2-, 4, 0.5-, M Phosphate 5, 0.2-, 6, 0.5M Chloride 7, Hydroxide

$[\alpha]_D$ + 140° (*c* 0.71) [Found: uronic anhydride (by decarboxylation), 36%] which contained the same sugar residues as acidic polysaccharide I. No residual polysaccharide was left in solution.

Fractionation of Acidic Polysaccharide II on DEAE-Cellulose

A sample (280 mg.) of acidic polysaccharide II was chromatographed on diethylaminoethylcellulose (30 g.) in a similar manner to acidic polysaccharide I. The accompanying diagram 2 shows the elution pattern. Again, five fractions were obtained, the major one (*ca* 70%), $[\alpha]_D$ + 150° (*c* 0.51) [Found: uronic anhydride (by decarboxylation), 40%] being eluted with 0.5M-phosphate buffer. The hydrolysis patterns of the fractions are found in table III.

TABLE III.

Eluant	Wt(mg.)	Sugars on Hydrolysis							
		G	Gal	Man	Ara	Xyl	Rha	Fuc	A.A.
0.05M-PO_4^{III}	19	-	+	++	tr	++	?	-	-
0.1M-PO_4^{III}	13	tr	+	+	+	++	+	+	++
0.5M-PO_4^{III}	143	tr	+	+	+	++	+	+	++
0.5M-Cl^1	17	tr	+	+	+	++	+	+	++
0.3M-OH^1	16	tr	+	+	+	++	+	+	++

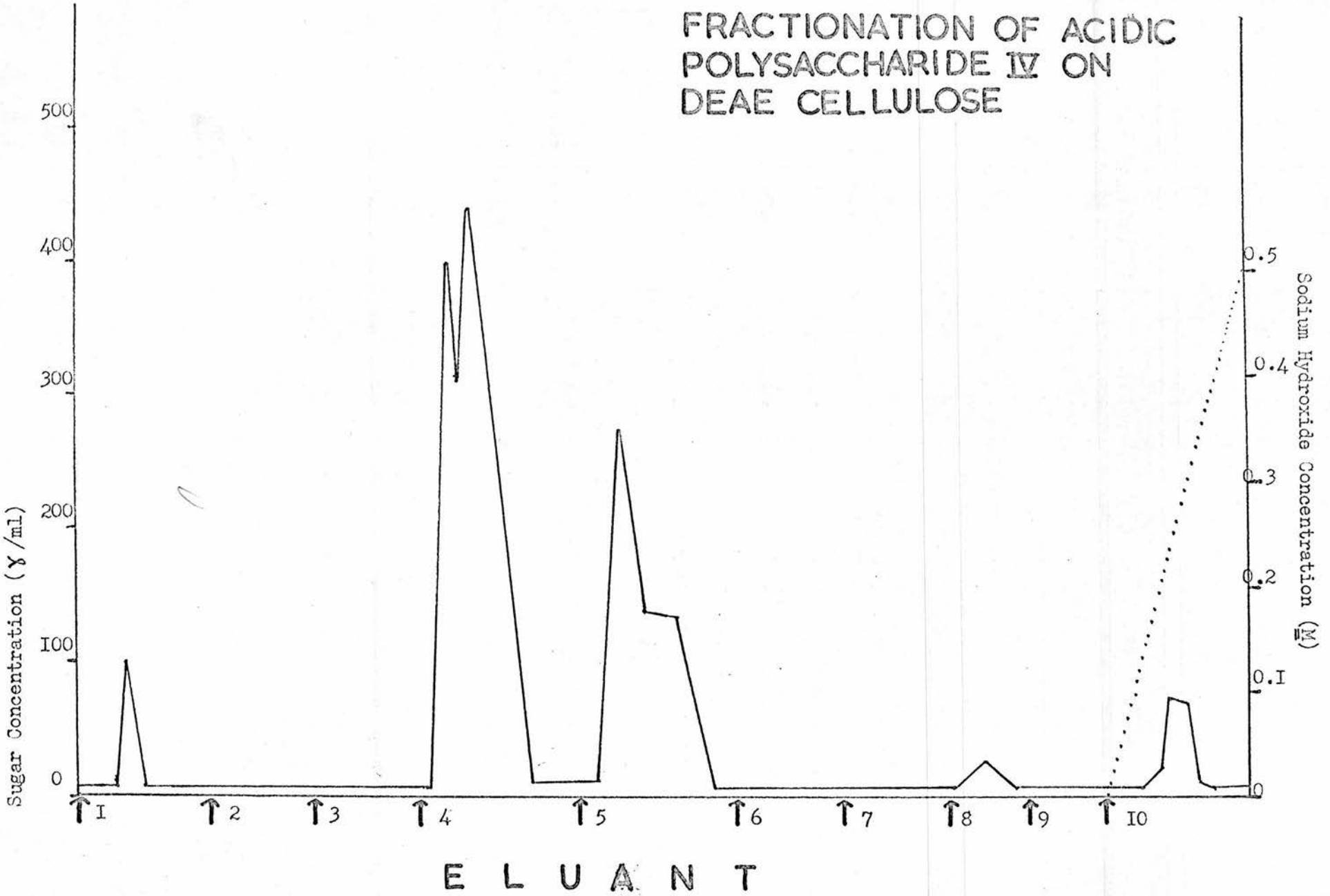
EDIA Extract.

Fractionation with Copper Acetate

The ethylenediaminetetra - acetic acid extract (29 g.) was dissolved in water (4l) and treated with 7% copper acetate solution (100 ml.) as previously described to give an acidic polysaccharide (9.2 g.), regenerated from its insoluble copper salt. Addition of sodium hydroxide (1 N : 100 ml.) gave a further precipitate which appeared to be galactomannan from its hydrolysis pattern. The acidic polysaccharide, after retreatment with copper acetate gave Acidic Polysaccharide IV* (7.8g.), $[\alpha]$ + 65° (c 0.5) [Found : uronic anhydride (by decarboxylation), 44%; OMc, < 1%]. On hydrolysis, the polysaccharide gave galacturonic acid, galactose, arabinose, xylose, and traces of rhamnose, fucose and two methylated sugars with the chromatographic mobility of 2-O - methylxylose and 2-O-methyl-fucose. When the supernatant liquid was treated with ethanol (1 vol.), a further polysaccharide fraction (0.58 g.) was isolated yielding arabinose and traces of xylose and galactose on hydrolysis.

* For Acidic Polysaccharide III, see reference 27.

DIAGRAM III


FRACTIONATION OF ACIDIC POLYSACCHARIDE IV ON DEAE CELLULOSE
Sugar Concentration (γ/ml)
500
400
300
200
100
0
Sodium Hydroxide Concentration (M)
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
ELUANT
I, 0.025- 2, 0.05- 3, 0.I- 4, 0.2- 5, 0.5-M Phosphate 6, 0.I- 7, 0.2- 8, 0.3- 9, 0.5-M Chloride I0, Hydroxide

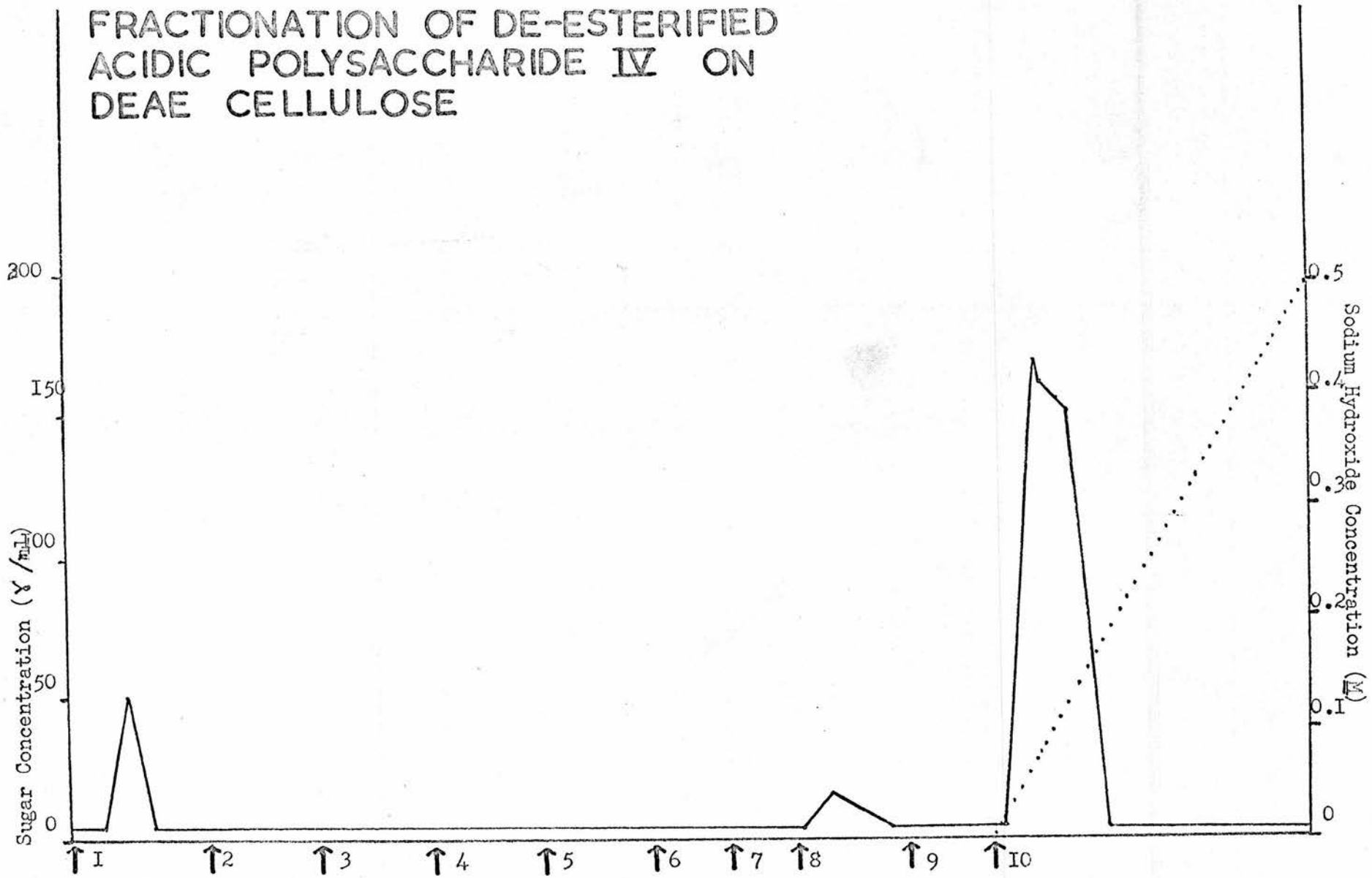
Fractionation of Acidic Polysaccharide IV on DEAE Cellulose

A sample (170 mg.) of acidic polysaccharide IV was chromatographed on diethylaminoethylcellulose under similar conditions as acidic polysaccharide I. Five fractions in all were obtained, the major two, eluted with 0.2 and 0.5M phosphate buffers, being essentially similar with $[\alpha]_D$ *ca* 100° and uronic anhydride (by decarboxylation), 45%. The accompanying diagram 3 gives the elution pattern while the yields and hydrolysis products are given in table IV.

TABLE IV

		Sugars on Hydrolysis						
Eluant	Wt(mg.)	G	Gal	Ara	Xyl	Rha	Fuc	A.A.
0.025M-PO_4^{III}	6	+++	-	-	-	-	-	-
0.2M-PO_4^{III}	87	tr	++	++	++	++	tr	++
0.5M-PO_4^{III}	40	tr	++	++	++	++	tr	++
0.3M-Cl^{1}	5	tr	-	-	++	-	-	-
0.1M-OH^{1}	12	tr	++	++	++	+	tr	++

DIAGRAM IV



I, 0.025- 2, 0.05- 3, 0.I- 4, 0.2- 5, 0.5M Phosphate 6, 0.I- 7, 0.2- 8, 0.3- 9, 0.5M Chloride I0, Hydroxide

Saponification of Acidic Polysaccharide IV.

A sample (50 mg.) was saponified by shaking overnight with 0.5N- sodium hydroxide (20 ml.) in the dark. The sodium salt was recovered by addition of ethanol (1 vol.) and dried by solvent exchange

Fractionation of Saponified sample on DEAE - Cellulose.

The above sample (47 mg.) was chromatographed as above on DEAE - cellulose. Only three fractions were obtained as shown in diagram 4, the major one (ca 90%) being eluted with sodium hydroxide. Yields and hydrolysis patterns are in table V.

TABLE V

Eluant	Wt(mg.)	Sugars on Hydrolysis						
		G	Gal	Ara	Xyl	Rha	Fuc	A.A.
0.025M-PO_4^{III}	2	+++	-	-	-	-	-	-
0.3M-Cl[1]	2	++	-	-	+++	=	-	-
0.1M-OH[1]	31	tr	++	++	++	++	tr	++

Hemicellulose 1.

Fractionation with Fehling's Solution

Hemicellulose 1 (51 g.) was steeped overnight in water (2 l.) and solubilised by the addition of sodium hydroxide (2N : 2 l.) Fehling's solution was added dropwise and with stirring, bringing about precipitation of the polysaccharide as its copper complex. The first part (ca 5%) of the precipitated complex was discarded as it contained mannose residues. The major portion of the complex was decomposed with ethanolic 1% hydrogen chloride and dried by solvent exchange to give Xylan A (35.6 g.), $[\alpha]_D$ - 93° (c 1.0 in N-NaOH) [Found : uronic acid(by decarboxylation), ca 4%]. Hydrolysis of the xylan gave xylose as the sole neutral sugar together with traces of glucuronic acid.

A small quantity of material (ca 1 g.) was left in solution and recovered by addition of ethanol (1 vol.). It appeared very similar to Hemicellulose 2, giving on hydrolysis galacturonic acid, galactose, arabinose, xylose and traces of rhamnose and fucose.

Hemicellulose 2.

Fractionation with Copper Acetate.

Hemicellulose 2 (14.2 g.), dissolved in water (1 l.), was treated with 5 ml. of 7% copper acetate solution. A large amount of precipitate was recovered and purified to give polysaccharide 2A(2.32 g.), $[\alpha]_D$ - 81.5° (c 0.37 in N-NaOH) [Found : uronic anhydride (by decarboxylation), ca 4%], containing xylose as the main sugar residue but also with traces of galactose and arabinose moieties.

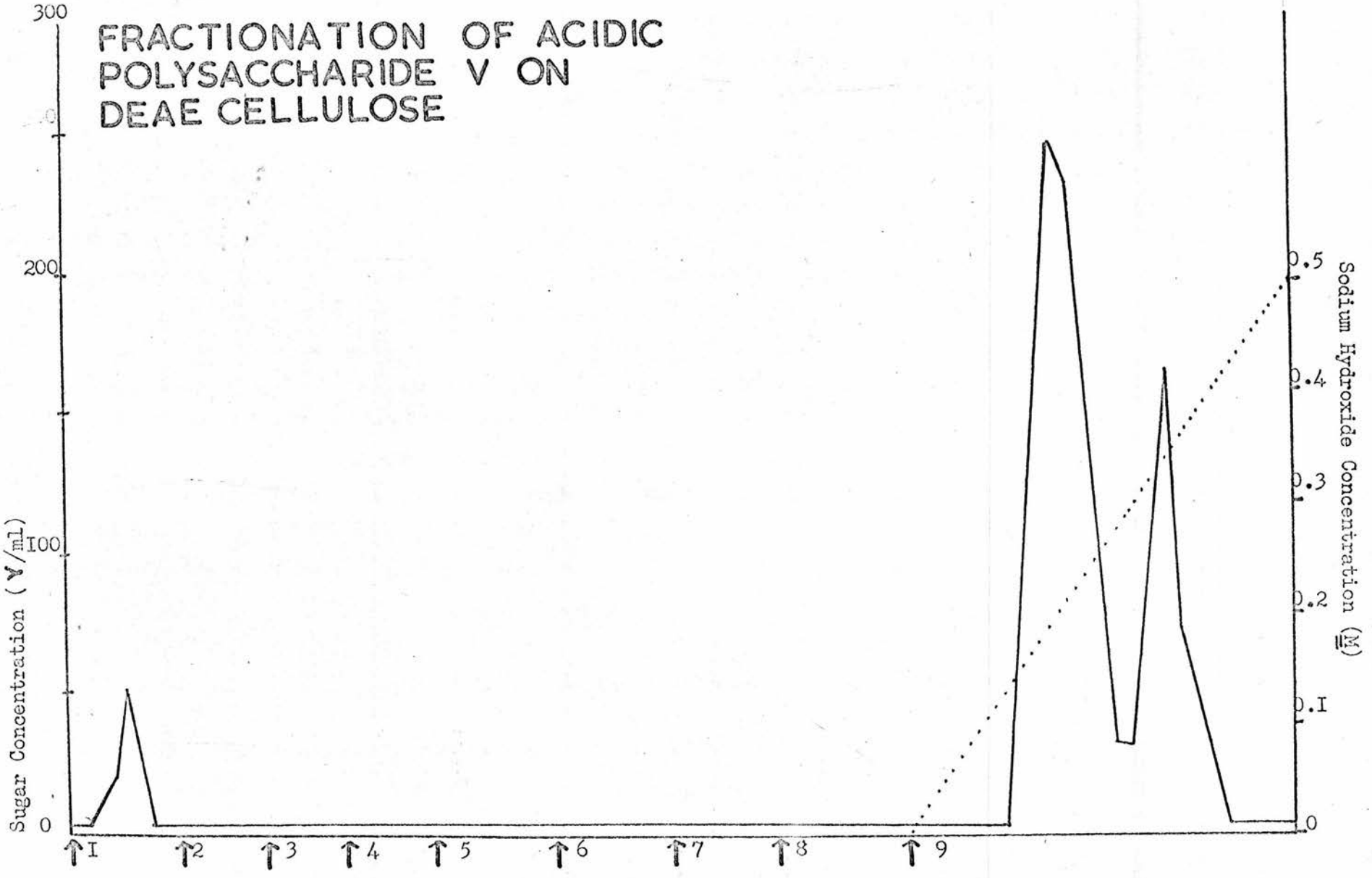
Addition of a further 95 ml. of copper acetate solution to the residual solution gave a further precipitate which yielded polysaccharide 2B (4.0 g.), $[\alpha]_D$ + 47°(c 0.48) [Found : uronic anhydride (by decarboxylation), 25%].

Addition of sodium hydroxide (2N : 100 ml.) to the supernatant solution gave a further polysaccharide fraction (1.3 g.) giving on hydrolysis xylose, glucose, galactose and traces of arabinose, rhamnose and galacturonic acid.

Relative Sugar Proportions in Polysaccharide 2B.

A sample (10 mg.) of polysaccharide 2B was hydrolysed with 2N-sulphuric acid at 100° for four hours. The individual (neutral) sugars present were estimated after paper chromatographic separation in appropriate solvents by

DIAGRAM V



I, 0.025- 2, 0.05- 3, 0.I- 4, 0.2- 5, 0.5M Phosphate 6, 0.I- 7, 0.2- 8, 0.5M Chloride 9, Hydroxide

Wilsons method (46). The presence of glucose, galactose, arabinose, xylose, fucose and rhamnose residues in the proportions of 1.5 : 3 : 4 : 11 : 1 : 3 was indicated.

Fractionation of Polysaccharide 2B on DEAE-Cellulose.

A sample of polysaccharide 2B (100 mg.) was chromatographed on diethylaminoethylcellulose in a similar manner to acidic polysaccharide I. Only two bands were obtained, the major one, $[\alpha]_D$ + 145° [Found : uronic acid anhydride (by decarboxylation) ca 40%], being eluted with sodium hydroxide. The elution pattern is found in diagram 5 while the yields and hydrolysis patterns are shown in table VI.

TABLE VI

Eluant	Wt.(mg.)	Sugars on Hydrolysis						
		G	Gal	Ara	Xyl	Rha	Fuc	A.A.
0.025M-PO_4^{III}	3	+++	-	tr	tr	-	-	tr
0.3M-OH^1	36	+	++	++	+++	+	tr	++

Relative Sugar Proportions in NaOH Eluted Polysaccharide.

A sample (10 gm.) of the sodium hydroxide eluted material was hydrolysed in the same way as 2B above. The proportions

of sugar residues were now found to be glucose, galactose, arabinose, xylose, fucose and rhamnose in the proportions of 1.5 : 3 : 4 : 5 : 1 : 3 as well as unestimated amounts of acidic sugars.

Fractionation of Polysaccharide 2B on Cellulose.

Polysaccharide 2B (3.8 g.) was dissolved in water (600 ml.) and chromatographed on a cellulose column (250 g.) which had previously been washed with 4% sodium hydroxide. The aqueous washings were collected and concentrated before Acidic Polysaccharide V was precipitated out with ethanol (1 vol.). The polysaccharide (2.7 g.), $[\alpha]_D$ + 140° (c 0.8) [Found : uronic anhydride (by decarboxylation), 45%] had a similar sugar content on hydrolysis to the sodium hydroxide eluted fraction from DEAE-cellulose.

The cellulose was extracted with 4% sodium hydroxide overnight and solubilised polysaccharide precipitated out by addition of ethanol (1 vol.). Hydrolysis gave xylose and glucose, the latter sugar presumably arising from degraded cellulose.

Hemicellulose 3.

Fractionation with N - Sodium Hydroxide.

Hemicellulose 3 (56 g.) was vigorously shaken over-

night with sodium hydroxide (N, 1 l.) in the dark and under nitrogen. The extracted polysaccharide 3A was recovered by addition of ethanol (1 vol.) and contained glucose, mannose and xylose residues in the approximate ratio of 3 : 2 : 2. The insoluble polysaccharide 3R (38 g.) gave only glucose on hydrolysis.

Hemicellulose 4

Fractionation with Fehling's Solution.

A solution of hemicellulose 4 (2.4 g.) in water (1,200 ml.) was made alkaline by addition of an equal volume of sodium hydroxide (2N.). Addition of freshly-prepared Fehling's solution resulted in two discrete fractions being obtained, the first, 4A with 5 ml. and the second (4B) with a further 45 ml. of reagent. Decomposition of the resultant complexes with ethanolic 1% hydrogen chloride gave polysaccharide 4A (1.14g.) which gave mainly mannose on hydrolysis and polysaccharide 4B(0.45 g.) which contained xylose as the main constituent sugar.

Fractionation of 4A and 4B with Cetyltrimethylammonium Hydroxide.

Polysaccharide 4A (1.10 g.) was dissolved in water (800 ml.) A solution of O.1N cetyltrimethylammonium hydroxide, prepared by passing a O.1M solution of cetyltrimethylammonium bromide through a column of Amberlite 1RA -

400 (OH) resin, was added dropwise and with stirring. The precipitated material was collected and dried by the usual methods. The precipitated polysaccharide (330 mg.) was found to contain mannose and xylose residues with traces of glucose and galactose. The residual solution was freed from cetyltrimethylammonium ions with Amberlite 1R120(H) resin and ethanol (1 vol.) added to give a "mannan" (626 mg.), $[\alpha]_D$-31° (c 0.25 in N-NaOH). Quantitative estimation of the constituent sugars by Wilson's Method (46) showed mannose, galactose and glucose residues in the ratio 23 : 2 : 1.

The polysaccharide 4B was similarly fractionated with cetyltrimethylammonium hydroxide solution. A very small amount of material was precipitated (ca 20 mg.) and appeared to be a xylan, giving only xylose on hydrolysis. The material remaining in solution was recovered as for the "mannan" but gave mannose and xylose on hydrolysis.

XYLAN A.

METHYLATION WITH DIMETHYL SULPHATE AND SODIUM HYDROXIDE.

Xylan A (10 g.) was steeped overnight in water (80 ml.) and solubilised by the addition of potassium hydroxide (60%, 170 ml.). The solution was kept at 0° and free from oxygen by passing nitrogen through while methyl sulphate (100 ml.) was added dropwise and with vigorous stirring over five hours. The solution was kept at 0° and stirred overnight. Thereafter, the solution was kept at room temperature and daily additions of methyl sulphate (100 ml.) and potassium hydroxide (170 ml.) were made dropwise over the next four days. After the third addition, the partially methylated xylan was kept in solution by adding sufficient acetone.

Excess acetone was removed and the cooled solution was neutralised with glacial acetic acid. Water (500 ml.) was added and the cooled solution was heated to the boiling point. Methylated polysaccharide separated out, was filtered off and the solution again heated to boiling to give further material. The precipitated material was dissolved in chloroform and the solution was dried over anhydrous sodium sulphate, concentrated and poured into light petroleum (B.P. $60\text{-}80^{\circ}$), giving partially methylated

xylan (7.5 g.) [Found : OMe, 32%].

METHYLATION WITH METHYL IODIDE AND SILVER OXIDE.

The partially methylated xylan (7.3 g.) was dissolved in methyl iodide (120 ml.) containing dry methanol (10 ml.) Silver oxide (25 g.) was added in portions of 2.5 g. per hour to the stirred and refluxing mixture in the dark. The solution was refluxed for a further fourteen hours and filtered. The silver salts were extracted with chloroform in a Soxhlet extractor and the combined solutions were concentrated to a syrup. The syrup was taken up in a small volume of chloroform and methylated polysaccharide precipitated by pouring into light petroleum (B.P. 60-80°) before drying over phosphorus pentoxide.

This procedure was repeated, omitting dry methanol, until no further increase in methoxyl content was noted (2 times) to give methylated xylan A (4.5 g.) [Found: OMe, 36.9%]. Fractional dissolution of the methylated polysaccharide in mixtures of chloroform and light petroleum (B.P. 60-80°) gave only one major fraction.

METHYLATION WITH METHYL IODIDE AND SILVER OXIDE IN N,N-DIMETHYL-FORMAMIDE.

The methylated polysaccharide was dissolved in methyl iodide (60 ml.) and diluted with an equal volume of N.N. dimethylformamide. Silver oxide (25 g.) was added and the mixture was vigorously shaken overnight. The supernatant liquid was filtered, excess methyl iodide removed by distillation under reduced pressure and N,N-dimethylformamide removed by repeated distillation under reduced pressure with toluene. The silver salts were continually extracted with chloroform and the solution combined with the residue from the filtrate. The fully methylated xylan A (3.4 g.), $[\alpha]_D$ - 71° [c 1.0 in chloroform] [Found: OMe, 38.9%] was precipitated as a white powder by pouring into light-petroleum (B.P. 60-80°) and drying over phosphorus pentoxide.

HYDROLYSIS OF METHYLATED XYLAN AND SEPARATION OF NEUTRAL AND ACIDIC SUGARS.

The methylated xylan (3 g.) was refluxed for six hours with methanolic 4% hydrogen chloride (220 ml.) until the optical rotation was constant. The solution was neutralised with silver carbonate, filtered and concentrated to a syrup (2.98 g.). The syrup was dissolved in hydrochloric acid

(0.5N, 200 ml.) before refluxing for eighteen hours. The cooled solution wasneutralised with silver carbonate, filtered, treated with hydrogen sulphide to precipitate residual silver salts, filtered and concentrated to a syrup (2.9 g.) This syrup was adsorbed on a column of diethylaminoethyl-Sephadex A-25 (30 g., formate form). Neutral methylated sugars were eluted by washing with water and concentrating to a syrup (2.5 g.). Acidic methylated sugars were recovered on elution with 0.5 N formic acid. This solution was concentrated to 60 ml., formic acid was removed with ether in a liquid-liquid extractor and the aqueous solution was fully concentrated to a syrup (90 mg.).

SEPARATION OF NEUTRAL SUGARS ON CHARCOAL-CELITE.

The syrup of neutral methylated sugars was adsorbed on a charcoal - Celite column (160 g., 1 : 1) and eluted by a linear gradient method from water through to 5% butan -2- one. The eluate was collected in 25 ml. fractions and every tenth tube was analysed by paper chromatography to determine the component sugars. In all, four fractions were obtained which are summarised in table VII.

TABLE VII

FRACTION	%BUTAN-2-ONE	WT.	R_G(SOLVENT E)	METHYLATED SUGAR
1	0.67 - 0.83	20mg.	0.51	Mono-Me-xylose
2	0.83 - 1.25	69 mg.	0.51	Mono-Me-xylose
			0.79	2,3,Me_2-xylose
3	1.25 - 2.30	2.01g.	0.79	2,3,Me_2-xylose
4	2.30 - 2.60	61mg.	0.79	2,3,Me_2-xylose
			0.93	2,3,4,Me_3-xylose

ANALYSIS OF FRACTIONS

Fraction 1.

Chromatography in solvent E gave only one spot but in solvent F, the fraction was resolved into two components, the faster one being chromatographically similar to 3-O-methylxylose and giving an orange-red colour with aniline oxalate spray, while the slower one was chromatographically similar to 2-O-methylxylose and gave a reddish-violet colour with the same spray.

Gas-liquid chromatography of the methyl glycosides gave peaks with the retention times of the following two sugars.

Sugar	Column A	Column B
2-O-methylxylose	4.16, 6.22	(0.96), 1.30
3-O-methylxylose	3.66, 5.60	1.11, (0.96)

Ionophoresis in borate buffer gave two spots, one of M_G = 0.70 being identical to 3-O-methylxylose and the other of M_G = 0.38 being similar to 2-O-methylxylose. The colours given by the sugars with the aniline oxalate spray were the characteristic orange-red and reddish-violet respectively.

The mixture of sugars was separated by thick-paper electrophoresis in borate buffer. Appropriate areas were eluted off with water and the buffer removed with Amberlite resins 1R 120(H) and 1R4B(OH), giving 2-O-methylxylose (5 mg.) and 3-O-methylxylose (12 mg.)

FRACTION 2

This fraction contained two components, one with the chromatographic mobility of 2,3 di -O-methylxylose in solvent E and a mono-methylxylose which was identified as 2-O-methyl-xylose by paper chromatography in solvent F and ionophoresis in borate buffer (M_G = 0.38).

Gas-liquid chromatography of the methyl glycosides gave peaks with the retention times of the following sugars.

Sugar	Columh A	Column B
2-O-methylxylose	4.16 6.2	0.97 1.27
2,3di -O- methylxylose	1.51 1.78	0.65 0.77

The sugars were separated by thick-paper chromatography in solvent E to give 2-O-methylxylose (16 mg.) and 2,3 di -O-methylxylose (28 mg.).

FRACTION 3.

This fraction was chromatographically pure and gas-liquid chromatography of the methyl glycoside gave only peaks corresponding to the retention times of 2,3 di -O-methylxylose.

Column A	Column B
1.51, 1.78	0.64, 0.75

FRACTION 4.

Paper chromatography of this fraction in solvent E showed the presence of two components, identical to 2, 3 di -O-methylxylose and 2,3,4 tri -O-methylxylose and thick paper separations in this solvent gave two chromatographically pure sugars, 13 mgs. and 40 mgs. respectively.

Gas-liquid chromatography of the methyl glycosides of the trimethylxylose fraction gave peaks with the retention times of the methyl glycosides of 2,3 di -O-methylxylose and 2,3,4 tri -O-methylxylose. Further hydrolysis of this fraction with 0.5N hydrochloric acid followed by separation of the components on filter sheets gave 2,3 di-O-methylxylose (14 mg.) and 2,3,4 tri -O-methylxylose (20 mg.) Gas liquid chromatography of the methyl glycosides of the trimethylxylose fraction gave peaks with the following retention times.

Sugar	Column A	Column B
2,3,4 tri -O-methylxylose	0.49, 0.62	0.46, 0.55

Characterisation of Sugars.

2-O-Methyl-D-xylose did not crystallise but was characterised by formation of N-phenyl -D-xylosylamine -2- monomethyl ether, m.p. and mixed m.p. 122-124°.

3-O-Methyl -D-xylose did not crystallise but was converted into 3-O-methyl -D-xylosazone, m.p. and mixed mlp. 170-171°.

2,3 Di -O-methyl -D-xylose crystallised on seeding and had m.p. and mixed m.p. 76-78°, $[\alpha]_D$ + 63° + 22° [Found : OMe, 34.5% (Calculated for $C_7H_{14}O_5$ = 34.8%)]. The sugar was further characterised as its N-phenylglycosylamine,

m.p. and mixed m.p. 120-123° and by conversion into 2,3 di -O-methyl -D-xylonamide, m.p. and mixed m.p. 132°-133°.

2,3,4 Tri -O-methyl -D-xylose did not crystallise but gave the aniline derivative, m.p. and mixed m.p. 99-101°.

Acidic Sugar

The acidic sugar (90 mg.), R_G 0.70 in solvent J, from the diethylaminoethyl-Sephadex column was freed from contaminating 2,3 di-O-methylxylose by thick paper separation. A portion of the sugar was converted into its methyl ester methyl glycosides by refluxing with methanolic 1% hydrogen chloride, reduced with lithium aluminium hydride in tetrahydrofuran and hydrolysed to give 2,3,4 tri -O-methylglucose and 3-O-methylxylose, identified by paper chromatography in solvents E and F, ionophoresis and gas-liquid chromatography of the derived methyl glycosides.

Methylation and Reduction of Methylated Aldobiouronic Acid.

The remainder (70 mg.) of the partially methylated aldobiouronic acid was dissolved in N:N-dimethylformamide (5 ml.) and shaken for 18 hrs. in the dark with silver oxide (2 g.) and methyliodide (5 ml.). After filtering and concentrating, a sample was methanolysed and gas-

liquid chromatography gave peaks with the retention times of the methyl glycosides of the indicated sugars.

Sugar	Column A	Column B
3,4 di -O-methylxylose	1.34, 1.62	0.74
2,3,4 tri -O-methyl glucuronic acid*	2.48, 3.20	1.76, 2.20

* as methyl ester.

The fully methylated aldobiouronic acid (68 mg.) was converted into its methyl ester methyl glycosides and dissolved in dry tetrahydrofuran (20 ml.). Lithium aluminium hydride (100 mg.) in tetrahydrofuran (10 ml.) was added dropwise with stirring. After 15 min. excess hydride was destroyed by adding ethyl acetate and water. A precipitate was filtered off before the neutral disaccharide was extracted with chloroform (3 x 40 ml.) and the extract was dried over anhydrous sodium sulphate before concentrating to a syrup. Gas-liquid chromatography of the methanolysis products gave peaks with the retention times of the methyl glycosides of 3,4 di -O-methylxylose and 2,3,4 tri -O-methylglucose.

Hydrolysis, Separation and Characterisation of Components.

The methylated disaccharide (44 mg.) was hydrolysed with 3% hydrochloric acid and the products were separated by filter sheet chromatography in solvent E. Two components were obtained, which were similar in chromatographic mobility to 2,3,4 tri -O-methylglucose (R_G= 0.89) and 3,4 di -O-methylxylose.

The faster moving sugar (12 mg.) was confirmed as 2,3, 4 tri -O-methyl -D-glucose by conversion into its aniline derivative, m.p. and mixed m.p. 144-146°.

The slower moving sugar (10 mg.) was oxidised to 3,4,-di-O-methyl -D-xylonolactone, m.p. and mixed m.p. 64-66°.

Molecular Weight of Methylated Xylan.

The molecular weight of the methylated xylan was measured as 9,500 ± 500 by the technique of vapour-phase osmometry (kindly measured by Mr. J.J. Carlyle.).

PARTIAL HYDROLYSIS OF XYLAN.

Xylan A (10g.) was thoroughly dispersed in 72% sulphuric acid (20 ml.) before diluting the solution to normal with water (500 ml.) This solution was heated on a boiling water bath until the rotation was constant (7 hr.) before cooling

and neutralising with barium hydroxide to pH5 and finally with barium carbonate. After filtering and deionising with Amberlite 1R120(H) resin, the solution was concentrated to a syrup (8.5 g.) which was adsorbed on a column of diethylaminoethyl - Sephadex A-25 (20 g. : formate form). Elution of the column with water furnished a syrup which, on paper chromatographic analysis, showed xylose and a sugar with the chromatographic mobility of 4-O-β-D-xylopyranosyl -D-xylose while elution with 0.5N formic acid, followed by removal of the formic acid with ether in a liquid-liquid extractor, gave a mixture of acidic oligosaccharides (280 mg.). These sugars were separated on filter sheets in solvent B, giving five fractions.

Fraction 1.(27 mg.) was chromatographically identical to glucuronic acid (R_{xyl} = 0.60).

Fraction 2.(2 mg.) was chromatographically indistinguishable from 2-O-(α-D-galactopyranosyluronic acid) -L-rhamnose and gave galacturonic acid and rhamnose on hydrolysis.

Fraction 3. (91 mg.) was chromatographically pure and identical to 2-O-(α-D-glucopyranosyl uronic acid) -D-xylose [R_{xyl}=0.28 (Solvent B)]. Hydrolysis of the sugar gave xylose and glucuronic acid. Hydrolysis of the reduced methyl ester methyl glycosides gave glucose and xylose in approximately equimolar amounts.

The aldobiouronic acid was methylated and the products of methanolysis, when examined by gas-liquid chromatography, gave peaks with the retention times of the methyl glycosides of the following sugars.

Sugar	Column A	Column C
3,4 di -O-methyl xylose	1.27, 1.55	1.07, 1.24
2,3,4 tri -O-methyl glucuronic acid, methyl ester	2.39, 3.07	2,31, 3.05

Fraction 4. (9 mg.) was chromatographically pure [R_{xyl}=0.09 (solvent B)] and gave glucuronic acid, xylose, glucurone and a trace of fraction 3 on hydrolysis. The derived methyl ester methyl glycosides were reduced with borohydride and hydrolysed to give glucose and xylose in the approximate ratio of 1 : 2.

Fraction 4 (5 mg.) was chromatographically pure [R_{xyl}= 0.02 (solvent B)] and gave glucuronic acid, xylose, glucurone and a trace of the sugar in fraction 3. Reduction with borohydride of the derived methyl ester methyl glycosides gave glucose and xylose in the approximate ratio of 1 : 3.

EXAMINATION OF MANNAN

Acetylation of Mannan

The mannan (151 mg.) was suspended in formamide (20 ml.) and acetylated as described in "General Procedures", using pyridine (20 ml.) and acetic anhydride (14 ml.) to give acetylated polysaccharide (204 mg.)

Methylation of Acetylated Polysaccharide.

The acetylated mannan was dissolved in tetrahydrofuran (40 ml.) and simultaneously methylated and de-acetylated by Hamilton and Kircher's method. Dry powdered sodium hydroxide (30 g.) and methyl sulphate (35 ml.) were added to the stirred mixture in tenth portions over four days. After the second addition, the contents were refluxed for one hour to complete deacetylation. As the mixture tended to become solid, more tetrahydrofuran was added to maintain fluidity.

On ending the additions, the mixture was refluxed for an hour to destroy methyl sulphate, cooled and filtered. The solid material was extracted with chloroform in a Soxhlet and combined with the filtrate. The solution was evaporated to dryness, taken up in a small volume of chloroform and poured into light petroleum (B.P. 60-80^{o})

to give partially methylated polysaccharide (120 mg.)

Methylation with Methyl Iodide and Silver Oxide.

Silver oxide (5 g.) was added in tenth portions to a solution of the partially methylated mannan in boiling methyl iodide (20 ml.) during four hours. After a further four hours refluxing, the mixture was filtered and the silver residues thoroughly washed with chloroform. The combined chrloroform washings and filtrate were concentrated, and methylated polysaccharide (44 mg.) was recovered by adding the solution to light petroleum (B.P. 60-80°) when methylated polysaccharide was precipitated as a white powder (Found : OMe, 41.2%).

Analysis of Methylated Polysaccharide.

A. Gas-Liquid Chromatography.

Methylated polysaccharide (2 mg.) was methanolysed with methanolic 3% hydrogen chloride. The resultant methyl glycosides gave peaks with the retention times of the following sugars.

Sugar	Column A	Column B	Relative Proportions
Unknown	-	1.06	+
2,3,4,6Me_4 mannose	1.40	1.32	+
2,3,4,6Me_4 galactose	1.80	1.61	+
2,3,6Me_3 glucose	3.60	-	tr
2,3,6Me_3 mannose	5.03	2.27	+++++++
2,3Me_2 mannose	-	3.24	+

B. Paper Chromatography

The major part of the methylated polysaccharide (38 mg.) was methanolysed as before and the methyl glycosides were hydrolysed with 3% hydrochloric acid. The mixture of methylated sugars was separated on filter sheets and each sugar was co-chromatographed in solvents E and K with authentic samples as well as giving the parent sugar on demethylation.

Methylated Sugar	Sugar on demethylation	R_G(E)	Proportion
2,3,4,6Me_4 mannose	mannose	1.07	+
2,3,4,6Me_4 galactose	galactose	0.96	+
2,3,6Me_3 glucose	glucose	0.87	+
2,3,6Me_3 mannose	mannose	0.84	+++++++
2,3Me_2 mannose	mannose	0.67	+
Mono Me mannose	-	0.43	tr

2,3,6 - Tri -O-methyl -D- mannose was characterised by conversion into the 1,4-di-p-nitrobenzoate, m.p. and mixed m.p. 187-189°.

Acetylation of Mannan.

The mannan (100 mg.) was acetylated as above to yield acetylated polysaccharide (104 mg.)

Acetolysis of Acetylated Mannan

To a stirred mixture of acetic acid (5 ml.), acetic anhydride (5 ml.) and concentrated sulphuric acid (0.5 ml.) acetylated mannan was added over a period of two hours. After leaving at room temperature for 72 hr., the solution was poured into water (100 ml.) and the pH adjusted to 5 with sodium bicarbonate. A small amount of precipitate was centrifuged off and extracted with chloroform (20 ml.) The centrifugate was also extracted with chloroform (4 x 20 ml.). The combined chloroform extracts were washed with sodium bicarbonate solution, dried and concentrated to a syrup. This syrup was dissolved in a mixture of methanol and chloroform (2 : 1; 15 ml.) before treating with 0.5N barium methoxide solution (2 ml.) to de-acetylate.

After leaving at 0^{o} for 20 hours, the mixture was poured into water, neutralised with dilute sulphuric acid, filtered and deionised with Amberlite IR 120 (H) and IR 4B(OH) resins to give a syrup (47 mg.)

Fractionation on Charcoal - Celite.

The above syrup was adsorbed on a charcoal - Celite column (1 : 1; 30 g.) which was washed with water to remove monosaccharides (mannose, glucose and galactose). Oligosaccharides were eluted by gradient method from 0-25% ethanol. Five ml. fractions were collected and every tenth tube was analysed bypaper chromatography. The elution pattern is given in the following table VIII.

TABLE VIII

FRACTION	ELUANT	WT(mg.)	R_{man}(A)	COMPONENT(*=major)
1	0 - 4%	3	0.50	O_1
2	4 - 5%	1	0.51	O_1 *
			0.34	Unknown
3	5 - 7%	2	0.51	O_1 *
			0.20	O_2
4	7 - 8%	1	0.51	O_1
5	8 - 9%	1	0.53	O_1?
			0.72	Unknown
6	9 - 15%	1	0.53	O_3
7	15 - 25%	1	0.21	O_2

Analysis of Fractions.

O_1 was identical to 4-O-β-D-mannopyranosyl -D-mannose on chromatographic and electrophoretic mobility (M_G, 0.64). Hydrolysis of the sugar gave only mannose as did hydrolysis of the derived glycitol.

O_2 was identical on paper chromatography to O-β-D-mannopyranosyl (1→4)-O-β-D-mannopyranosyl (1→4) -D-mannose and also had similar ionophoretic mobility. Hydrolysis gave only mannose while partial hydrolysis gave mannose and O_1.

O_3 was chromatographically and ionophoretically similar to 4 -O-β-D-glucopyranosyl -D-glucose and gave only glucose on hydrolysis.

ACETOLYSIS OF POLYSACCHARIDE 3R.

Polysaccharide 3R (5 g.) was cooled with acetic anhydride (14 ml.) in an ice-salt mixture. A similarly cooled solution of concentrated sulphuric acid (2.8 ml.) in acetic anhydride (6 ml.) was added and the mixture was macerated to an even paste with a glass rod. Formation of a paste was enhanced by heating to 60° for a few minutes before leaving the reddish mass at room temperature for 75 hr. Acetylated oligosaccharides in chloroform were isolated as described previously under "Acetylation of Acetylated Mannan" and de-acetylation was also completed as previously noted.

Paper chromatography of the sugars showed only three components, glucose, cellobiose and cellotriose, which were finally separated into these three components on filter sheets.

Characterisation of Oligosaccharides.

Cellobiose (220 mg.) was pure chromatographically [R_G = 0.50(A)] and ionophoretically (M_G = 0.21). Hydrolysis gave only glucose while hydrolysis of the

derived glycitol also gave glucose as the only reducing sugar. The sugar crystallised from ethanol - water, m.p. and mixed m.p. 221-223, $[\alpha]_D$ + 26.5 → + 37.8° (equib.) (C 0.4) and was further characterised for formation of its α octa-acetate, mp. and mixed m.p. 226 - 228°.

Cellotriose (98 mg.) was chromatographically (R_G = 0.29[A]) and ionepheretically pure and gave only glucose on hydrolysis. Partial hydrolysis gave glucose and cellobiose. The sugar crystallised from ethanol - water, m.p. and mixed m.p. 203-206° (dec.) and gave the characteristic α- hendeca-acetate, m.p. and mixed m.p. 221-222°.

Acetolysis of Resistant Residue.

A sample (10 g.) of the residual material after all the extractions had been carried out was subjected to graded acetolysis, using an analogous procedure to that used for polysaccharide 3R, only scaling up the quantities. After deacetylation, a quantity.

of material precipitated out during concentration. This powder (0.5 g.) gave only glucose on hydrolysis and is probably a mixture of higher cellodextrins. The syrup (6.9 g.) obtained from complete concentration was applied to a charcoal - Celite column (150 g ; 1 : 1) and monosaccharides were eluted with water (glucose plus traces of mannose, galactose and xylose). Elution by stepwise increase in ethanol concentration gave the following table IX of results.

TABLE IX

FRACTION	ELUANT (%ETHANOL)	WT (mg.)	R_G (A)	Component (*=major. tr=trace)
1	O - 1%.	88	*	Glucose *
				Mannose
			0.48	O_1

FRACTION	ELUANT	WT.	R_G (A)	COMPONENTS.
2	1%	53	0.45	O_1
			0.50	O_2
3	2 - 5%	1,500	0.50	O_2
4	5%	22	0.50	O_2 *
			0.37	Unknown
5	7.5%	79	0.50	O_2 *
			0.82	Unknown
6	10-12.5%	400	0.29	O_3
7	15%	28	0.29	O_3 *
			0.22	O_4

Fractions were further separated on filter sheets where necessary.

Analysis and Characterisation of Oligosaccharides.

Oligosaccharide O_1 (12 mg.) was chromatographically identical to 4 - O -β-D -mannopyranosyl -D-mannose and gave only mannose on hydrolysis. Ionophoretic mobility was also similar (M_G = 0.64).

Oligosaccharide O_2 (1.6 g.) was chromatographically and ionophoretically indistinguished from 4 -O-β-D-glucopyranosyl -D- glucose and gave only glucose on

hydrolysis. This sugar crystallised from ethanol-water with m.p. and mixed m.p. 223-224°, $[\alpha]_D$ + 26.1° → + 36.8° (equil.) (c 1.0) and was characterised as cellobiose by formation of its α-octa-acetate, m.p. and mixed m.p. 226-227°, $[\alpha]_D$ + 44.7° (c 0.5 in $CHCl_3$).

Oligosaccharide O_3 (0.4 g.) had chromatographic and ionophoretic mobilities of O-β-D-glucopyranosyl (1→4) -O-β-D-glucopyranosyl -(1→4) -D-glucose and gave glucose only on hydrolysis while partial acid hydrolysis gave glucose, cellobiose and a trace of unhydrolysed sugar. The sugar crystallised from ethanol-water with m.p. and mixed m.p. 203-204° (dec.), $[\alpha]_D$ + 32° (equil.) (c 0.5). Cellotriose -α-hendeca-acetate was prepared, m.p. and mixed m.p. 221-223°, $[\alpha]_D$ +20° (c 0.2 in $CHCl_3$).

Oligosaccharide O_4 (4 mg.) was chromatographically and ionophoretically similar to O-β-D-mannopyranosyl-(1→4) -O-β-D-mannopyranosyl -(1→4) -D-mannose and gave mannose only on hydrolysis. Partial acid hydrolysis gave mannose, mannobiose and original sugar.

Further Fractionation of Acidic Polysaccharides I and II.

As ion-exchange chromatography of acidic polysaccharides I and II still showed traces of neutral polysaccharide(s) as contaminants, they were each (1 g.) further fractionated on diethylaminoethylcellulose (phosphate form, 100 g.) Elution with 0.025M-phosphate buffer gave the neutral fractions while elution with 0.5M-phosphate gave pure acidic polysaccharides IA and IIA.

IA - Yield, 731 mg., $[\alpha]_D$ + 148° (c 1.1)

IIA - Yield, 684 mg., $[\alpha]_D$ + 142 (c 1.4).

Partial Acid Hydrolysis with N - Sulphuric Acid.

Samples of acidic polysaccharides I, II, IV and V (c 100 mg.) were hydrolysed with N-sulphuric acid (25 ml.) at 100°. Samples removed at intervals showed that acidic oligosaccharides were produced in maximum yield between 4 and 6 hours when examined by paper chromatography. Acidic oligosaccharides were adsorbed on DEAE-Sephadex A-25 (formate form), the columns were washed well with water and acidic sugars were recovered by elution with 0.5N-formic acid. The major sugars produced are shown in table X below.

Partial Acid Hydrolysis with N/2 - Sulphuric Acid.

Samples of acidic polysaccharides IA, IIA, and IV (c 100 mg.) were hydrolysed with N/2 - sulphuric acid (25 ml.) at 100°. Paper chromatographic evidence showed that neutral oligosaccharides were produced in maximum yield when hydrolysis had taken place between 2 and 2.5 hr. The major oligosaccharides formed are shown in table X below.

Partial Acetolysis

Samples of acidic polysaccharide IA, IIA and V (c 500 mg.) were acetylated as described in "General Procedures". The acetylated polysaccharide was dissolved in acetic acid (5 ml.) acetic anhydride (5 ml.) and concentrated sulphuric acid (0.5 ml.). Samples were removed at intervals and worked up as described in previous acetolysis experiments. The maximum yield of oligosaccharides was produced after 72 hrs. and the major sugars are listed in the table X below.

TABLE X.

HYDROLYTIC EXPERIMENT	ACIDIC POLYSACCHARIDE.							
	I OR IA		II OR IIA		IV		V	
	R_G VALUES IN GIVEN SOLVENT							
N-SULPHURIC ACID	B	C	B	C	B	C	B	C
	0.79(a)	0.75(a)	0.79(a)	0.75	0.81(a)	0.75(tr)	0.81(a)	0.75(tr)
	0.18(b)	0.52(b)	0.18(b)	0.32(b)	0.20(b)	0.53(b)	0.20(b)	0.53(b)
	0.08(c)	0.25(c)	0.08(c)	0.25(c)	-	0.20(c)	-	0.20(c)
N/2-SULPHURIC ACID	A	D	A	D	A	D	A	D
	0.51(d)	0.54(d)	0.51(d)	0.54(d)	-	0.51(tr)	-	-
	0.41(e)	0.43(e)	0.41(e)	0.44(e)	0.44(c)	0.40(e)	-	-
	0.24(tr)	0.29(tr)	0.24(tr)	0.29(tr)	0.31	0.27(tr)	-	-
	-	0.18(f)	-	0.18(f)	0.17	0.17(f)	-	-
ACETOLYSIS	B	D	B	D			B	D
	1.06(g)	1.18(g)	1.06(g)	1.18(g)	-	-	1.06(g)	1.18(g)
	1.60(h)	0.78(h)	0.60(h)	0.78(h)	-	-	0.60(h)	0.78(h)
	0.31	0.41	0.31	0.41	-	-	0.31	0.41
	0.10	0.21	0.10	0.21	-	-	0.10	0.21

tr = trace

The above oligosaccharides had the chromatographic mobilities of the following sugars:-

a. 2-O-(α-D-Galactopyranosyluronic acid)-L-rhamnose.
b. 4-O-(α-D- galactopyranosyluronic acid) -D-galactopyranosyluronic acid.
c. Polymer homologous trigalacturonic acid.
d. 4-O-β-D-mannopyranosyl -D-mannose.
e. 4-O-β-D-galactopyranosyl -D-galactose.
f. Polymer homologous mannotriose and galactotriose.
g. 2-O-α-L-fucopyranosyl -D-xylose.
h. 2-O-β-D-galactopyranosyl -D-xylose.

PARTIAL ACID HYDROLYSIS OF COMBINED ACIDIC POLY-SACCHARIDES I & II.

Combined acidic polysaccharides I & II (8 g.) were hydrolysed as one for six hours with N-sulphuric acid (400 ml.). A small quantity of material (130 mg.) separated out during hydrolysis and was removed at the centrifuge. Total hydrolysis of this material gave galacturonic acid, rhamnose and a trace of xylose. Addition of acetone (1 vol.) to the supernatant liquid gave a further precipitate (47 mg.) which was found to contain the same sugar residues as thefirst precipitate. The solution was concentrated to remove acetone and the pH was adjusted to 5 by adding saturated barium hydroxide solution before filtering. Neutralisation was finally achieved by shaking the solution with methyldi-n-octylamine (5% v/v in chloroform). The solution was passed through a column of Amberlite IR120(H) resin before concentrating to a syrup (6.9 g.)

This syrup was applied to a diethylaminoethyl-Sephadex-A25 column (40g.; formate form) and neutral sugars (3.5 g.) were recovered by eluting the column with water. These included galactose, arabinose, xylose,

fucose, and rhamnose as well as traces of oligosaccharides. Elution of the column with water containing increasing amounts of formic acid gave 10 fractions which were further separated, where necessary, by filter sheet chromatography giving the following major oligosaccharides.

Oligosaccharide 1. (471 mg. ; eluted with 0.05 N formic acid). R_{galA}=0.78 (Solvent B), M_G = 0.54, $[\alpha]_D$ = +89° (c 4.5) gave galacturonic acid & rhamnose only on hydrolysis and was chromatographically and electrophoretically similar to 2-O-(α-D-galactopyranosyluronic acid) -L-rhamnose. The sugar was methylated with methyl sulphate and sodium hydroxide solution, the solution was acidified and the sugar was extracted with chloroform. The methylated disaccharide was crystallised and recrystallised from a mixture of chloroform-light petroleum (B.P. 100-120°), and had m.p. and mixed m.p. 67-69° (Kofler hot-stage microscope), $[\alpha]_D$ = +99.3° [c 2.1 in chloroform]. The crystals gave an X-ray powder photography identical to that given by methyl 2-O-(2,3, 4 tri -O- methyl -α-D-galactopyranosyluronic acid) -3, 4 di-O- methyl -L- rhamnose dihydrate.

Oligosaccharide 2 (25 mg. ; eluted with 0.05 N formic acid), R_{galA} = 0.19 (Solvent B), = 0.56 (Solvent C),

M_G = 1.04, $[\alpha]_D$ = +1° (c 1.3) was chromatographically and electrophoretically indistinguishable from 6-O-(β-D-glucopyranosyluronic acid) -D-galactose. Hydrolysis of the aldobiouronic acid gave glucuronic acid, galactose and glucurone while the derived methyl ester methyl glycosides, when reduced with borohydride, gave glucose and galactose on hydrolysis. The derived glycitol gave glucuronic acid as the only reducing sugar on hydrolysis. Methanolysis of the methylated disaccharide gave major peaks with the retention times of the methyl glycosides of the following sugars.

Sugar	Column A	Column B
2,3,4 tri-O-methyl galactose	7.18	2.65, 2.90
2,3,5 tri-O-methyl galactose	4.35	1.97
2,3,4 tri -O-methyl-glucuronic acid*	2.44, 3.15	1.79, 2.22

* as methyl ester.

Oligosaccharide 3 (8 mg.; eluted with 0.05 N formic acid), R_{galA}=0.27(Solvent B), = 0.70 (Solvent C), M_G = 0.67, $[\alpha]_D$ = -16° (c 0.31) was contaminated with a trace of oligosaccharide 2 but had the chromatographic and

ionophoretic mobility of 4-O-(β-D-glucopyranosyluronic acid) -D-galactose and gave glucuronic acid, galactose and glucurone on hydrolysis. The derived methyl ester methyl glycosides were reduced with borohydride and gave glucose and galactose on hydrolysis. Hydrolysis of the glycitol derived from the aldobiouronic acid gave only glucuronic acid as a reducing sugar.

The mixture was converted to its methyl ester methyl glycosides and reduced with borohydride before methylating. Gas-liquid chromatography of the methanolysis products gave peaks with the retention times of the methyl glycosides of the following sugars.

Sugar	Column A	Column B
2,3,4,6 tetra -O-methylglucose	1.00, 1.38	1.00,1.41
2,3,6 tri -O-methylgalactose	3.03,(4.17),4.55	1.58,2.54
2,3,5 tri -O-methylgalactose (tr)	(4.17)	-
2,3, 4 tri -O-methylgalactose (tr)	6.95	-

tr = trace.

Oligosaccharide 4 (39 mg.; eluted with 0.05N formic acid), R_{galA} = 0.34 (Solvent B), =0.80 (Solvent C), M_G =0.84, $[\alpha]_D$ = -16° (c 0.79) was chromatographically similar to 2-O-(β-D-glucopyranosyluronic acid) -D-mannose. The sugar gave glucuronic acid, mannose and glucurone on hydrolysis

while the borohydride reduced methyl ester methyl glycosides gave glucose and mannose on hydrolysis. The derived glycitol gave only glucuronic acid as a reducing sugar on hydrolysis.

The methylated aldobiouronic acid was reduced with lithium aluminium hydride in tetrahydrofuran. The methanolysis products from the resultant neutral disaccharide were examined by gas-liquid chromatography and gave peaks with the retention times of the methyl glycosides of the following sugars.

Sugar	Column A	Column B
2,3,4 tri -O-methylglucose	2.56, 3.71	1.36, (1.79)
3,4,6 tri -O-methylmannose	3.02	(1.79)

The aldobiouronic acid gave no colour with the triphenyl-tetrazolium spray.

Oligosaccharide 5 (14 mg. ; eluted with 0.05 N formic acid), R_{galA} = 0.53 (Solvent B), = 0.93 (Solvent C), M_G = 0.60, $[\alpha]_D$ = -61° (c 1.1) was chromatographically and ionophoretically indistinguishable from 4-O-(β-D-glucopyranosyl-uronic acid) -L-fucose. The sugar gave glucuronic acid, fucose and glucurone on hydrolysis while the borohydride reduced methyl ester methyl

glycosides gave glucose and fucose on hydrolysis. The glycitol derived from the aldobiouronic acid gave glucuronic acid as the sole reducing sugar. Gas-liquid chromatography of the methanolysis products from the methylated sugar gave peaks with the retention times of the following sugars:-

Sugar	Column A	Column B
2,3 di-O- methylfucose	1.06, 1.51	0.62,0.88,0.97
2,3,4 tri -O-methyl glucuronic acid*	2.44, 3.15	1.81, 2.25

* as methyl ester.

Oligosaccharide 6 (84 mg. ; eluted with 0.05 - 0.4N formic acid) R_{galA}= 0.12 (Solvent B), = 0.57 (solvent C), M_G = 0.65, $[\alpha]_D$ + 96° (c 1.4) contained galacturonic acid and rhamnose residues in the molar ratio of 1.1 : 1 (40, 42), while the derived glycitol contained these sugar residues in the ratio of 2.1 : 1. Mild acid hydrolysis of the tetrasaccharide gave mainly 2-O-(α-D-galactopyranosyluronic acid) -L-rhamnose while similar treatment of the glycitol obtained by borohydride reduction gave this some sugar, a trace of rhamnitol and a major sppt which separated from 2-O-(α-D-galactopyranosyluronic acid) -L- rhamnose in solvent H and had a similar

mobility to 2-O-(α-D-galactopyranosyluronic acid) -L-rhamnitol.

The methylated tetrasaccharide was methanolysed and examined by gas-liquid chromatography to give peaks with the retention times of the methyl glycosides of the following sugars.

Sugar	Column A	Column B
3,4 di-O-methylrhamnose	0.98 - 0.99	0.61
2,3,4 tri -O-methyl - galacturonic acid*	7.08	3.87, 4.23
2,3, di -O-methyl - galacturonic acid*	5.2	2.21-2.22

* as methyl ester

The derived glycitol was also methylated and the methanolysis products examined by gas-liquid chromatography. Peaks were obtained which corresponded to the methyl glycosides of the following sugars.

Sugar	Column A	Column B
3,4, di -O-methylrhamnose	0.98-0.99	0.60
1,3,4,5 tetra -O-methylrhamnitol	1.07	0.78
2,3 di -O-methyl - galacturonic acid*	5.2	2.23
2,3, 4 tri -O-methyl - galacturonic acid*	7.05	3.88,4.26

*as methyl ester.

Gas-liquid chromatography of the methanolysis products after the methylated tetrasaccharide had been reduced with lithium aluminium hydride in tetrahydrofuran gave peaks with the retention times of the methyl glycosides of 3, 4 di-O-methylrhamnose and 2,3, 4 tri-O-methylgalactose but no 2,3, di -O-methylgalactose. This last sugar was identified by paper chromatography of the hydrolysis products of the carboxyl reduced methylated tetra-saccharide on multiple development in solvent M.

Oligosaccharide 7 (28 mg. ; eluted with 0.4N formic acid) R_{galA} = 0.12 (Solvent B), = 0.45 (Solvent C), M_G = 0.88, $[\alpha]_D$ = +80° (c 1.4), contained galacturonic acid and rhamnose residues in the molar ratio of 1.9 : 1 (40, 42) while the derived glycitol contained no rhamnose. On mild acid hydrolysis of trisaccharide only traces of

galacturonic acid and rhamnose were released. The derived methyl ester methyl glycosides were reduced with potassium borohydride and gave galactose and rhamnose in the ratio of 2 : 1 (Visual). This neutral trisaccharide was methylated with methyl sulphate and sodium hydroxide and the methanolysis products were examined by gas-liquid chromatography. Peaks were obtained which corresponded in retention times to the methyl glycosides of the following sugars.

Sugar	Column A	Column B
3, 4 di -O-methylrhamnose	0.97	0.56
2,3,6 tri -O-methylgalactose	3.10,4.18,4.50	(1.57)2.26,2.51
2,3,4,6 tetra -O-methyl galactose	1.72	(1.57)

Oligosaccharide 8 (41 mg.; eluted with 0.5 N formic acid) R_{galA}=0.52 (Solvent C), M_G = 0.95. This sugar gave galacturonic acid only on hydrolysis and had similar chromatographic and ionophoretic mobility to 4-O-(α-D-galactopyranosyluronic acid) -D-galacturonic acid. The sugar (30 mg.) in water (5 ml.) was neutralised with calcium carbonate, filtered and the calcium salt precipitated with acetone. After drying, the white powder had $[\alpha]_D$ + 118° (c 0.57 in 0.5N hydrochloric acid).

Oligosaccharide 9 (61 mg.; eluted with 0.5N formic acid)

R_{galA}= 0.24 (Solvent C), M_G = 0.95. This sugar gave only galacturonic acid on hydrolysis and was chromatographically and electrophoretically indistinguishable from O-α-D-galactopyranosyluronic acid -(1→4)-O-α-D-galactopyranosyluronic acid -(1→4) -D-galactopyranosyluronic acid. The calcium salt was isolated as for digalacturonic acid and gave $[\alpha]_D$+ 134° (c 0.91 in 0.5N hydrochloric acid).

ACETOLYSIS OF ACIDIC POLYSACCHARIDE IV.

Acidic polysaccharide IV (5.5 g.) was acetylated as described in "General Methods" giving acetylated polysaccharide (6.1 g.). This material was dispersed thoroughly in acetic acid (160 ml.) and acetic anhydride (160 ml.) and concentrated sulphuric acid (16 ml.) was added dropwise and with vigorous stirring over two hours. Stirring was continued for a further three hours before leaving the solution at room temperature for a further seventy-five hours. After about 50 hrs., a scum appeared which increased in quantity and was removed by centrifugation.

Hydrolysis of the material gave galacturonic acid, rhamnose and traces of xylose. The supernatant solution was poured into ice-water (500 ml.) and centrifuged. The residue was extracted with chloroform and the supernatant solution was brought to pH 3 with sodium bicarbonate before extracting it, also with chloroform (4 x 100 ml.) The combined chloroform extracts were dried over anhydrous sodium sulphate and concentrated to a syrup. The syrup was taken up in chloroform (10 ml.) and methanol (20 ml.) and deacetylated with 0.5N - barium methoxide (20 ml.). After leaving at 0° for 24 hrs., the solution was poured into water, neutralised with dilute sulphuric acid, filtered, barium ions removed with Amberlite

1R 120(H) resin, filtered and concentrated to a syrup (2.7 g.)

The syrup was adsorbed on a diethylaminoethyl-Sephadex-A25 column (20 g. ; formate form) and elution with water, followed by concentration, furnished a syrup of neutral sugars (1.2 g.). This syrup was applied to a charcoal-Celite column (60 g.: 1 : 1) and monosaccharides (600 mg.), including galactose, arabinose, xylose and fucose were recovered by elution with water. Elution of the column with water containing increasing quantities of ethanol gave several oligosaccharides which were further separated on filter sheets if necessary.

Elution of the DEAE-Sephadex column with water containing increasing quantities of formic acid gave several acidic oligosaccharides which were also further separated on filter sheets.

Acetolysis of Acidic Polysaccharide V.

Acidic polysaccharide V (1.5 g.) was acetylated, subjected to graded acetolysis, separated into neutral and acidic components and these components further separated into individual sugars as described in "Acetolysis of Acidic Polysaccharide IV" above.

Acetolysis of Soybean Meal Acidic Polysaccharide Complex.

The acidic polysaccharide complex (15 g.) from soybean meal (for extraction, fractionation and physical constants, see J.N.C. Whyte - Ph.D. Thesis (Edin). 1964), was subjected to graded acetolysis in a similar manner to acidic polysaccharide IV and gave a number of acidic and neutral oligosaccharides. The neutral components are summarised in table XI below.

TABLE XI.

Component	R_G(A)	M_G	A.P. IV		A.P. V		Meal.	
			Wt(mg)	$[\alpha]_D$	Wt(mg)	$[\alpha]_D$	Wt(mg)	$[\alpha]_D$
2-O-Me Xylose	2.10	-	8	-	3	-	107	-
2-O-Me Fucose	2.55	-						
Oligosaccharide 1	0.07	-	-	-	-	-	15	-
" 2	0.42	0.49	50	+87°(c1.1)	12	+71°(c1.2)	330	+83°(c 2.5)
" 3	0.60	0.73	-	-	-	-	45	-
" 4	1.28	0.44	-	-	-	-	38	+9° (c 0.38)
" 5	0.80	0.50	39	-30°(c1.2)	14	-40°(c1.4)	75	-36°(c 0.8)
" 6	1.16	0.38	32	-45°(c1.6)	9	-42°(c0.9)	26	-59°(c 0.5)
" 7	1.30	0.08	-	-	-	-	53	-50°(c 0.5)
" 8	0.20	0.40	8	+60°(c0.6)	2	-	200	+61°(c 0.9)
" 9	0.84	0.91	-	-	-	-	13	-
" 10	0.41	-	-	-	-	-	7	-
" 11	0.11	0.32	2	-	-	-	106	+48°(c 1.0)
" 12	0.44	Solvent G -		-	-	-	323	-
" 13	0.31							
" 14	0.24							

The mixtures of 2-O-methylxylose and 2-O-methylfucose was separated on filter sheets and each component was further identified by gas-liquid chromatographic examination of the derived methyl glycosides.

Oligosaccharide 1 (R_G 0.07) gave arabinose, xylose and rhamnose on hydrolysis but was not examined further.

Oligosaccharide 2 (R_G 0.42) gave galactose on hydrolysis while the derived glycitol gave galactose and galactitol on hydrolysis. Gas-liquid chromatography of the methanolysis products from the methylated sugar gave peaks with the retention times of the methyl glycosides of 2,3,4,6 tetra -O-methylgalactose and 2,3,6 tri -O-methylgalactose. The sample from acidic polysaccharide IV crystallised from ethanol-water and had m.p. and mixed m.p. 201-204° and $[\alpha]_D$ + 90° → + 57° (Equil.)(c 2.1). The sample from the meal complex also crystallised from ethanol-water and had m.p. and mixed m.p. 204° and $[\alpha]_D$ + 95° (5 min.)→+ 63° (18 hr.) (c 1.68). Both crystalline sugars gave X-ray powder photographs identical to that obtained from an authentic sample of 4-O-β-D-galactopyranosyl -D-galactose.

Oligosaccharide 3 (R_G, 0.60) gave galactose on hydrolysis. The sugar gave a mauve colour with the hydroxylamine - ferric chloride spray and after treatment with 1% ammonia for 18 hrs. gave a spot similar in chromatographic mobility to galacto-

biose.

Oligosaccharide 4 (R_G 1.28) gave arabinose only on hydrolysis while the derived glycitol gave arabinose and arabitol on hydrolysis. The ratio of arabinose in the oligosaccharide to arabinose in the glycitol was 2.2 : 1, based on phenol-sulphuric acid measurements. The chromatographic mobility of the benzylglycosylamine derivative in solvent L (R_F = 0.67) also suggested the sugar was a disaccharide. The sugar did not release any formaldehyde on oxidation with periodate. The gas chromatograms obtained from the methanolysis products from the methylated sugar, methylated glycitol and methylated aldonolactone could not be fully interpreted but showed by the presence in all cases of methyl 2,3,5 tri -O-methyl-arabinosides that non-reducing arabinofuranose end units were present.

Oligosaccharide 5 (0.80) gave galactose and xylose on hydrolysis and galactose and xylitol were obtained by hydrolysis of the derived glycitol. The sugar gave no colour with the triphenyl-tetrazolium spray. The gas chromatograms from the methanolysis products of the methylated disaccharides contained major peaks with the retnetion times of the methyl glycosides of 2,3,4,6 tetra-O-methylgalactose and 3,4di-O-

methylxylose. The fully methylated disaccharide from the meal complex was hydrolysed and separated on filter sheets in solvent E into the two components:-

(i) 2,3,4,6 tetra -O-methyl -D-galactose (18.5 mg.), $[\alpha]_D$ + 128° (c 0.93) was converted into its aniline derivative, m.p. and mixed m.p. 195-197°.

(ii) 3,4 di -O-methyl -D-xylose (26 mg.), $[\alpha]_D$ + 16° (c 1.3) was converted into the crystalline 3,4 di -O-methyl -D-xylonolactone, m.p. and mixed m.p. 64-66°.

Oligosaccharide 6 (R_G 1.16) contained fucose and xylose residues while the derived glycitol gave fucose and xylitol on hydrolysis. The sugar gave no colour with the triphenyltetrazolium spray. The methanolysis products from the methylated sugar were identified by gas-liquid chromatography as the methyl glycosides of 2,3,4 tri -O-methylfucose and 3,4, di -O-methylxylose.

Oligosaccharide 7 (R_G 1.30) contained arabinose residues only while the derived glycitol gave arabinose and arabinitol on hydrolysis. The ratio of arabinose in the oligosaccharide to arabinose in the glycitol, based on phenol-sulphuric acid measurements, was 1.35:1. The chromatographic mobility of its benzylglycosylamine derivative in solvent L (R_F 0.62) also suggested it was a trisaccharide. The sugar did not release

any formaldehyde on periodate oxidation. Although the gas chromatograms from the methanolysis products of the methylated sugar and the methylated glycitol could not be fully interpreted, the presence of methyl 2,3, 5 tri -O-methylarabinofuranosides showed that non-reducing arabinofuranose end groups were present.

Oligosaccharide 8 (R_G 0.20) gave only galactose on hydrolysis while partial acid hydrolysis gave galactose and galactobiose.

A fully authenticated sample of O-β-D-galactopyranosyl (1→4) -O-β-D-galactopyranosyl-(1→4)-D-galactose obtained by partial acid hydrolysis of soybean meal acidic polysaccharide complex (68) was acetylated with acetic anhydride in pyridine. The resultant crystalline hendeca-acetate, probably β, had m.p. 212-213° and $[\alpha]_D$ + 27.5° (c 1.82 in chloroform).[Calc. for $C_{40}H_{54}O_{27}$:C, 49.7; H, 5.6 : Found: C, 49.42; H, 5.48%].

A sample of oligosaccharide 8 was similarly acetylated and the crystalline hendeca-acetate had m.p. and mixed m.p. 212-213°.

Oligosaccharide 9 (R_G 0.84) gave galactose, xylose and arabinose on hydrolysis but was not examined further.

Oligosaccharide 10 (R_G 0.41) gave only galactose on hydrolysis but gave a mauve colour with the hydroxylamine-ferric chloride

spray. Treatment with ammonia (1%) for 18 hr. gave a spot of similar chromatographic mobility to galactotriose.

Oligosaccharide 11 (R_G 0.11) gave only galactose on hydrolysis but gave galactose, galactobiose and galactotriose on partial acid hydrolysis. The sugar was methylated and the methanolysis products were examined by gas-liquid chromatography. Peaks were obtained with the retention times of the methyl glycosides of 2,3,4,6 tetra - and 2,3,6 tri -O-methylgalactose.

The sugar was acetylated with acetic anhydride in pyridine and gave its crystalline tetradeca-acetate, m.p. 131-135° (dec.)

Oligosaccharides 12, 13 and 14 (R_G 0.44, 0.31 and 0.24 in solvent G). The mixture gave only galactose on hydrolysis while partial acid hydrolysis gave galactose, galactobiose, galactotriose and galactotetraose. The methanolysis products from the methylated mixture, when examined by gas-liquid chromatography, gave peaks with the retention times of the methyl glycosides of 2,3,4,6 tetra- and 2,3,6 tri -O-methylgalactose. Oligosaccharide 12 was separated on filter sheets and acetylated with acetic anhydride in pyridine.

The crystalline heptadeca-acetate had m.p. 112-114° (dec).

The acidic oligosaccharides obtained from the acetolysis of the three polysaccharides are shown in table XII.

TABLE XII.

Component	R_{GalA}	A.P. IV		A.P.V		Meal.	
		Wt(mg)	$[\alpha]_D$	Wt(mg)	$[\alpha]_D$	Wt(mg)	$[\alpha]_D$
Acidic Oligosaccharide 1	0.78	46	-	6.5	$+85^{\circ}$(c 0.7)	42	-
" " 2	0.42-0.44	14	$+57^{\circ}$(c 0.9)	-	-	27	$+60^{\circ}$(c 0.3)
" " 3	0.07-0.09	10	$+80^{\circ}$(c 1.0)	-	-	18	$+90^{\circ}$(c 0.6)
" " 4	0.13	-	-	-	-	12	-

R_{GalA} in solvent B.

Acidic Oligosaccharide 1 (R_{GalA} 0.78) gave galacturonic acid and rhamnose on hydrolysis and the derived glycitol gave only galacturonic acid on hydrolysis. The methylated aldobiouronic acid was methanolysed and examined by gas-liquid chromatography. Peaks were obtained with the retention times of 3,4, di -O-methyl-rhamnose and 2,3,4 tri -O-methylgalacturonic acid. The sample of methylated aldobiouronic acid from acidic polysaccharide IV crystallised from chloroform - light petroleum (B.P. 100-120°) and had m.p. and mixed m.p. 67-70°, $[\alpha]_D$ + 83° (c 0.46 in $CHCl_3$). The similar sample from the meal complex also crystallised and had m.p. and mixed m.p. 67-70°, $[\alpha]_D$+90° (c 0.87 in $CHCl_3$). Both samples gave X-ray powder photographs identical to that from methyl 2-O-(2,3,4 tri -O-methyl -α-D-galactopyranosyluronic acid)-3,4 di -O-methyl -L-rhamnose dihydrate.

Acidic Oligosaccharide 2 (R_{GalA} 0.42-0.44). This sugar contained galacturonic acid and rhamnose residues in the molar ratio of 1.0 : 1.9 The derived glycitol contained the same residues in the ratio 1 - 1.1 : 1. Partial acid hydrolysis gave rhamnose and 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose while similar hydrolysis of the glycitol gave rhamnitol and the same aldobiouronic acid. The methanolysis

products from the methylated sugar gave peaks with the retention times of the methyl glycosides of 3,4 di -O-methylrhamnose and 2,3,4 tri -O-methylgalacturonic acid. The methanolysis products from the methylated glycitol obtained from acidic oligosaccharide 2 from acidic polysaccharide IV contained the methyl glycosides of the above two sugars and also a peak with the retention time of 1,3,4,5 tetra -O-methylrhamnitol.

The periodate - Schiff spray gave a yellow spot with the sugar from the meal complex and a blue one with its derived glycitol.

Acidic Oligosaccharide 3 (R_{GalA} 0.07-0.09). This sugar contained galacturonic acid and rhamnose residues in the ratio of 1-1.2 : 1 and gave only 2-O-(α-D-galactopyranosyluronic acid)-L-rhamnose on partial acid hydrolysis. The derived glycitol contained the same residues in the ratio of 2-2.1 : 1 and gave 2-O- (α-D-galactopyranosyluronic acid)-L-rhamnose and 2-O-(α-D-galactopyranosyluronic acid) -L-rhamnitol on partial acid hydrolysis. These two components separated on paper chromatography in solvent H.

The gas-chromatograms from the methanolysis products from the methylated tetrasaccharide gave major peaks with the retention times of the methyl glycosides of 3,4 di -O-methyl-

rhamnose, 2,3, di- and 2,3,4 tri -O-methylgalacturonic acid. The glycitol obtained from this sugar from acidic polysaccharide IV was methylated and the same glycosides as above were identified in the methanolysis products as well as 1,3,4,5 tetra -O-methylrhamnitol.

The sugar from the meal complex gave a yellow colour with the periodate-Schiff spray while its derived glycitol gave a blue spot.

Acidic Oligosaccharide 4 (R_{GalA} 0.13) contained galacturonic acid and rhamnose residues in the ratio of 1.1 : 1 while the derived glycitol gave the same sugars in the ratio of 1.9 : 1. Partial acid hydrolysis of the sugar gave rhamnose and a spot with the mobility of digalacturonosylrhamnose obtained from partial acid hydrolysis of acidic polysaccharides I and II. Similar treatment of the glycitol gave rhamnitol and the same acidic sugar. The sugar gave a yellow halo with the periodate-Schiff spray while its glycitol gave a blue spot.

The methanolysis products from the methylated tetrasaccharide were examined by gas-liquid chromatography and peaks were obtained with the retention times of the methyl glycosides of 3,4 di-O-methylrhamnose, 2,3 di- and 2,3,4 tri-O-methylgalacturonic acid.

METHYLATION STUDIES

Acidic Polysaccharide IV.

The methyl ester of acidic polysaccharide IV (500 mg.) was prepared by stirring a suspension of the polysaccharide in ether with an ethereal solution of diazomethane at 0° for 18 hrs, filtering, washing with ether and drying in air.

The methyl ester was swollen overnight in dimethylsulphoxide (20 ml.) and the solution diluted with an equal volume of N, N-dimethylformamide. The flask was placed in an icebath and the contents stirred. Crushed barium hydroxide (30 g.) was added and methyl sulphate was added dropwise over four hours. The ice bath was then removed but the stirring was continued. Fresh portions of methyl sulphate (5 ml. each) were added after 24 and 36 hr. and the mixture was stirred for a further 12 hr. An equal volume of water was now added and the solution was dialysed against tap water until all the barium ions had been removed. The residual solution was partially concentrated and then freeze-dried to give partially methylated polysaccharide (220 mg.).

The partially methylated polysaccharide was dissolved in N, N-dimethylformamide (10 ml.), methyl iodide (10 ml.)

and silver oxide (5 g.) added and the mixture vigorously shaken in the dark for 24 hrs. After filtering, the silver salts were well washed with chloroform, concentrated and combined with the residue obtained when the filtrate was evaporated to dryness by repeated distillation under reduced pressure with toluene.

This whitish solid residue was methylated as above and the methylated polysaccharide (80 mg.) was recovered by pouring a solution of it in a small volume of chloroform into a large excess of light petroleum (B.P. 60-80°) [Found: OMe, 37.2%].

The rather gummy precipitate was refluxed with light petroleum (B.P. 60-80°) containing increasing quantities of chloroform for 30 min(Total volume=30 ml.). The major portion of the polysaccharide (49 mg.) $[\alpha]_D$ + 102° (c 0.6 in $CHCl_3$) [Found : OMe, 39.6%] was soluble in light-petroleum:chloroform (80:20) and was recovered as a buff precipitate.

Acidic Polysaccharide V.

Acidic polysaccharide V (500 mg.) was converted to its methyl ester and methylated as described for acidic polysaccharide IV. The major portion of methylated polysaccharide, soluble in light-petroleum:chloroform

(80:20) gave a whitish precipitate (33 mg.) $[\alpha]_D$ + 133° (c 1.0 in $CHCl_3$) [Found OMe, 39.3%].

EXAMINATION OF CLEAVAGE PRODUCTS.

Samples (5 mg.) of the above two methylated polysaccharides were methanolysed with methanolic 4% hydrogen chloride. The products were examined by gas-liquid chromatography of the methyl glycosides and peaks were obtained with the retention times of the methyl glycosides of the following sugars. The relative proportions of each sugar are given in the following table.

Sugar	A.P.IV	A.P.V
2,3,4Me_3 Xylose	+++	+++
2,3,5Me_3 Arabinose	+	+
2,3,4Me_3 Fucose	+++	+++
2,3,4,6Me_4 Galactose	+++	+++
3,4,Me_2 Xylose	++++	++++
2,3,6Me_3 Galactose	+++	+++
2,3,Me_2 Arabinose	+	+
3,4Me_2 Rhamnose	+++	+++
3 Me Rhamnose	+	+
2,3Me_2 Galacturonic Acid*	10+	5+
2,3,4 Me_3 Galacturonic Acid*	+++	++

* as methyl ester.

A further sample of each methylated acidic polysaccharide (10 mg.) was methanolysed and the resulting mixtures of methyl glycosides were dissolved in dry tetrahydrofuran, reduced with lithium aluminium hydride in dry tetrahydrofuran. After 20 min., excess hydride was destroyed with ethyl acetate and water before extracting the neutral methyl glycosides with chloroform. The resultant syrups were hydrolysed with 4% hydrochloric acid and examined by paper chromatography in various solvents and paper ionophoresis. The following sugars were identified other than those already identified by gas-liquid chromatography.

	A.P.IV	A.P.V
2,3,4 Me_3 Galactose	+	+
2,3, Me_2 Galactose	++++	+++
2 Me Galactose	+	+
3 Me Galactose	+	+
Galactose	tr	tr

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